

ever, other instances of parasitism in the same class which have not received a proportionate amount of attention. The Epi-
 caridan Isopods are a case in point. That the Bopyrids and their
 allies induce modifications in the host comparable to those
 caused by Rhizocephala has been known to zoologists ever since
 Giard made clear in 1888 that the assertion of Rathke that only
 female Palaemons were parasitized by Bopyrus was
 accounted for by parasitic castration of the male. Little,
 however, seems to be recorded beyond the bare facts. Such
 information as is available is summed up in a few pages of
 Bonnier's monograph on the Bopyrids, in which some general
 observations on the changes caused by the parasites are given,
 with somewhat fuller details (and figures) of the modifications
 of the appendages of *Galathea intermedia* parasitized by
B. p. a. With this partial exception the subject, so
 far as it is aware, has never been seriously explored,
 although the conditions of para-
 sitism are sufficiently different
 of the Rhizocephala to suggest that
 be instructive. This neglect has
 the fact that as a rule the Epi-
 caridians are erratic in their occurrence and consequently difficult to obtain
 in sufficient numbers for the purpose of a detailed investigation.
Gyge branchialis at Naples is not subject to this draw-
 back. On the contrary the comparatively large percentage of
Upogebia which are parasitized by it render these species
 peculiarly favourable for investigation.

Most of the material was collected and the preliminary work
 on the externals carried out during the writer's tenure of the
 Oxford Biological Scholarship at Naples in 1924. Since then the
 work has been carried on, with certain more or less prolonged
 interruptions, in the Zoological Department at Cambridge and
 (for the larger part) in the Department of Zoology and Com-
 parative Anatomy at Oxford. A small amount of supplementary
 material was obtained during visits to Naples in the autumn
 of 1925, spring 1927, and autumn 1928.

Particular and grateful acknowledgement is made to Mr.
 E. B. Ford, B.Sc., for the most helpful assistance and advice

in the handling and interpretation of the data relating to chela growth. Various aspects of the subject and sundry special points connected with it have been discussed at different times with Dr. E. J. Allen, Dr. J. R. Baker, Prof. Julian Huxley, and Mr. Richard Palmer, to all of whom, and especially to Prof. Huxley, the writer's thanks are tendered, while he is also indebted to Prof. Goodrich for kindly reading the manuscript and making some helpful comments and suggestions. It is also a pleasure to thank Dr. R. Dohrn and the staff of the Naples Zoological Station for the facilities there provided and for the most ready helpfulness on all occasions.

II. THE BOPYRIDÆ.

As a background for the account which follows of the effects of *Gyge* on its host *Upogebia* a brief sketch of the organization and life-history of the parasite may not be out of place. *Gyge branchialis*, *Cornalia* and *Panceri*, is a member of the family Bopyridæ of the Isopoda Epicarida, all of which are parasitic in the branchial chamber of Decapod Crustaceans. This particular form and the initial stages of its development were carefully described and figured by the authors just named as long ago as 1858, from *Upogebia* collected at Venice. Occupying the gill-chamber of the host the parasite causes a characteristic swelling of the branchiostegite similar to that which the more familiar *Bopyrus* causes in *Palaemon*. Like other Bopyrids *Gyge* is a purely external parasite, merely sucking the juices of the host by means of its modified piercing and suctorial mouth-parts. Normally a male and female are always present together, and these exhibit a striking sexual dimorphism. The large female individual is asymmetrical, the head being slightly deflected either to the right or to the left according to the parasite's position on the right or left side of the host. The anterior end of the animal is always directed towards the host's posterior extremity. The flattened dorsal surface is turned towards the host's body. The mouth-parts are modified for piercing and sucking and there are seven short pereopods terminating in a hooked claw. From the bases of

the first five pairs there arise broad incubatory lamellae or oostegites, forming a brood-pouch for the eggs. The short abdominal region bears six pairs of reduced pleopods and a pair of small uropods. The minute and less highly modified male is carried near the posterior end of the body of the female.

The full life-history has not been followed out in this particular genus, but the larval stages show great uniformity in all the Bopyridae, and it will be convenient here to outline briefly the typical life-history found in the group. The young larva is released from the shelter of the incubatory lamellae of the parent in a form known as the *Epicaridan* larva, resembling in general appearance an ordinary, small, free-living isopod. There are short, three-jointed antennules, large antennae, six subchelate thoracic appendages, and six abdominal appendages. The mouth-parts have already assumed the special form found in the adult, namely, piercing, styliform mandibles working in a suctorial oral cone formed by upper and lower lips, the first maxillae being absent and the second maxillae vestigial.

There follows, at least in some forms, probably in many, and perhaps in all, a phase of temporary parasitism on pelagic copepods. The larva fixes on the copepod host and undergoes a moult. After this the integument remains soft, so that the appendages show no definite segmentation, the musculature undergoes a more or less considerable atrophy, the pleopods lose their setae, and some other minor changes occur. This is the *Microniscus* stage, long regarded as a distinct genus, the recognition of its true nature being mainly due to Sars (1899, pp. 218-20) and to Caullery (1907).

The *Microniscus* stage is followed by the *Cryptoniscus* stage, with the full seven pairs of thoracic limbs, rigid chitin, and fully segmented appendages. The *Cryptoniscus* larva is adapted to pelagic life. It leaves the temporary host to seek out and attach itself to the permanent decapod host. Having achieved this object it passes into the Bopyroid stage, characterized by a shortening up of the thoracic limbs, the great reduction of the antennules, antennae, and pleopods, the loss of the natatory setae of the latter, and the partial or complete abortion of the eyes. In this stage the minute male becomes

sexually mature. The female passes on to assume the characteristic form and organization of that sex.

The ordinary course of events following fixation appears to be as follows: The first larva to attach itself to the host obtains a liberal supply of nourishment from the juices of the latter and develops directly into a female, rapidly accommodating its size to that of the branchial chamber in which it has established itself. Subsequently another larva arrives, which takes up its position on the body of the first-comer, as already described, and becomes a functional male. According to Bonnier (1900) additional *Cryptoniscus* larvae may be present, at any rate in some forms, but only the first of these develops into the male; the remainder fail to metamorphose.

Nothing seems to be known concerning the factors directing development after fixation. The fact that increase in size is so regulated that the female always just fills the branchial cavity may be supposed to be due to growth being in some way inhibited by a certain degree of contact with or pressure against the walls of the cavity. Precisely how, if at all, the minute male feeds and what determines its development into a male instead of a female, together with various other questions connected with the life of the Bopyrids, is really not at all clearly or adequately understood, as I have emphasized in a recent letter to 'Nature' (1929), to which those interested are referred. The points there raised, however, are not directly relevant to the main subject of the present paper and need not be discussed further here.

III. GENERAL OBSERVATIONS ON *UPOGEBIA* AND *GYGE*.

Upogebia littoralis (Risso), one of the *Thalassinidea*, is common at Naples in shallow water where the bottom consists of muddy sand in which the animals make their burrows. I propose at some future time to publish a few observations on the habits and mode of burrowing of this species, but these need not detain us now. It may be mentioned that in most respects they are very much the same as those of *Calocaris macan-*

dreae, a functionally hermaphrodite Thalassinid from deep water, which have been carefully described by Runnström (1925) in a paper primarily concerned with the sexual organization of this remarkable form, to which further reference will be made later. The agreement which evidently exists between these two genera even in many details of behaviour and mode of operation in burrowing is rather striking in view of the considerable differences in organization and habitat.

A. Some Data on the Numbers of Host and Parasite.

As already stated *Gyge branchialis* is at the present time a comparatively common parasite of *Upogebia littoralis* in the Bay of Naples. In 1924 out of more than a thousand individuals of *Upogebia* examined 21.5 per cent. were parasitized by *Gyge*. The numbers were, however, by no means constant throughout the period of study (March to August), but on the contrary varied considerably in the different months. They rose from about 20 per cent. in March to over 25.5 per cent. in May, dropped again in June and July, reaching the minimum of about 15.5 per cent. in the latter month, and then rose again in August to about 23 per cent. In the absence of data for the other months it is hardly desirable to attempt to draw any conclusions from these figures. The apparent fluctuations may perhaps be merely due to local differences in the abundance of the parasite at different spots. Smaller collections made during subsequent shorter visits also vary quite considerably in the number of individuals parasitized. In the material obtained in late August and early September 1925 the proportion was as low as 17 per cent., while in the first three weeks of September 1928 it was as high as 31.4 per cent. This considerable difference in the numbers obtained at almost the same season of different years seems to favour the supposition that the variation observed is mainly or wholly due to accidents of collecting and not significant of any real seasonal fluctuation. At any rate there is no evidence to show that *Gyge* is liable to those sensational fluctuations in numbers which have been observed in some other *Epicarids*—e.g. *Ione thoracica*,

which was formerly abundant at Naples on *Callianassa*, but is now very scarce.

There are also some points of interest in connexion with the numbers of the *Upogebia*. In the extensive material examined there is a marked inequality in the sex ratio, females being the more numerous. In the large number collected in the summer of 1924 they outnumbered the males by 3 to 2. Here again it is probable that the observed numbers may convey a misleading impression. I am disposed to believe that they indicate not, as might at first sight be assumed, a real preponderance of females in nature, but rather, if one may use the expression, a differential susceptibility to the methods of capture employed. The *Upogebia* are collected by means of a long, narrow-bladed spade, which is dug into the mud, and the spadefuls of mud are then passed through a coarse sieve (or a finer one when very small specimens are wanted) in order to obtain the animals. If ovigerous females tend to remain nearer the mouths of the burrows than the males, as they might not unreasonably be expected to do for the better aeration of the eggs, the preponderance of females in the catches would be accounted for, and I believe that this is in fact the most probable explanation. It gains some support from the fact that in March, before the eggs were laid, the numbers of the sexes in the catches were approximately equal, though the total examined (113 individuals) was not very large.

In view of this excess of females in the unparasitized specimens it is at first sight somewhat surprising to find that amongst the parasitized specimens males outnumber females (55.6 per cent. ♂♂ to 44.4 per cent. ♀♀). It may be remarked at once that there can be no question of any sex-reversal having taken place under the influence of the parasite, since the nature of the modifications induced is such that if any such reversal occurred it would be in the direction from male to female and not from female to male. Any idea of the parasite showing a preference for one sex over the other may also, I think, be dismissed as very highly improbable. Some other explanation must be sought.

Now it is necessary to bear in mind here that the large majority of individuals, or in all probability practically all, are

parasitized when they are very young (see p. 10); and the explanation of the difference in the numbers of the sexes observed amongst parasitized individuals is therefore to be sought in the conditions obtaining during the early period of the animals' post-larval existence.

In point of fact, if the quite young animals of about 3–6 mm. carapace length (which have been left out of consideration up to now) are considered alone it is found that amongst these, just as among parasitized specimens of all ages, males do preponderate. Not much significance is to be attached to the exact figures which I obtain—about 61 per cent. males to 39 per cent. females—because the sample which I have counted is small, namely, about fifty individuals only, but it is sufficient to note that the excess of males is quite definite.

It is consequently safe to say that the excess of males amongst parasitized *Upogebia* is simply a reflection of an effective excess of males at the period when parasitization normally takes place. This excess might be explained in two ways. Either there is a real inequality in the sex-ratio, males being rather the more numerous, only this is masked in the mature individuals by some such difference in the reactions of the sexes during the breeding season as I have suggested, or else the sex ratio is equal, but there is some difference in behaviour between young males and young females which renders the former more liable both to the attacks of the parasite and to capture by the method already described. That such a difference in the behaviour of the two sexes should exist in these very young animals—a difference, be it noted, whose effect on the proportions of males to females in the catches would be just the reverse of that found in the case of mature specimens—appears to me unlikely.¹ I am inclined to believe that there is a real inequality

¹ It is perhaps worth remarking that even supposing some such difference existed it could hardly be of the same kind as postulated in the case of adult animals. If the supposed tendencies for the females to remain nearer the mouths of the burrows and the males deeper down were reversed during early life—in itself improbable—this would still probably have no effect on the numbers of males and females collected, since probably the maximum depth of the burrows of these small specimens is well within the average depth of a spade thrust.

in the sex ratio, but I do not stress this very strongly since the evidence is hardly adequate for the formation of a definite opinion.

B. The Period of Fixation of the Parasite.

In most species of Epicarids quite young individuals of the host species are the most commonly parasitized. In the case of *Gyge* Bonnier remarks that the *Gebia* are 'surtout infestées dans le jeune âge'. For my own part I am fully convinced that it is the normal, if not indeed the invariable, rule for infestation to take place while the *Upogebia* is still *very young* and for host and parasite to develop *pari passu*. This is consistently indicated by several lines of evidence. I have never found an adult *Upogebia* bearing a parasite in which the latter did not fill the branchial chamber, and I have examined many scores of parasitized individuals. If fixation on adult *Upogebia* were at all frequent—or in fact anything but very rare—one would expect, however quickly the parasite grows after establishing itself, to find at least a few cases in which fixation was so recent that the parasite had not had time to reach the full size appropriate to that of the host. Again, I have not found in any but the smallest specimens cases of males in which the female abdominal appendages which appear under the influence of the parasite were not of their full size relative to the total size of the animal. It is, normally at any rate, only in quite small parasitized males that one finds these organs in the actual process of development following recent parasitization. In the larger and older parasitized individuals they are always, in my experience, fully developed. In other words one does not find in these latter animals any cases where the evidence would indicate recent fixation of the Epicarid.

To define the period of fixation rather more precisely, the evidence indicates that this usually takes place when the host is under 17 mm. total length. One may find individuals of 16 and 17 mm. (carapace length about 6–6.5 mm.) in which parasitization is so recent that the appendages have not yet begun to develop, but I have not met with any of a larger size than this

in which they were still absent or even very small. A single individual (male) from which I have obtained the *Cryptoniscus* larva of *Gyge* is about 16 mm. long (carapace length 6.5 mm.). It shows no signs of female appendages.

The measurements of chelae in normal and parasitized animals which are discussed in the section devoted to this subject will be found to give a result in harmony with the above. In the graphs based on the mean chela measurements for individuals of given carapace lengths the divergence between the curves for parasitized and normal males occurs in the region of about 7-8 mm. carapace length.

The conclusion which would be drawn from these graphs considered alone would be that the normal period of parasitization is before the attainment of the length just named. Since a little time must be allowed for the influence of the parasite to become visibly reflected in the size of the chelae the further inference would be justified that the upper age-limit for fixation of the parasite on at least the majority of the infected animals is appreciably below the said point. Further, since the divergence between the normal and parasitized male curves after the critical point is considerable, it must be supposed that individuals parasitized after reaching this size either do not occur or are so very few that they are insufficient to raise the average chela measurements anywhere near the normal male value (see pp. 23 *et seq.*). These deductions are in complete agreement with the conclusions previously reached.

C. The Length of Life of Host and Parasite.

In those groups of *Epicarida* in which the sexually mature female is so profoundly modified and degenerate that it perishes after reproduction, the animals may be practically confined to comparatively young individuals of the host species. This is not the case, however, with the *Bopyridae*, for here the changes undergone are much less considerable, and the female certainly breeds more than once. Though it is evident in the case of *Upogebia* and *Gyge* that parasitization normally takes place while the host is quite small, the parasite is still alive and

vigorous after a sufficient time has elapsed for the host to attain its full size.

With regard to the length of life of the host it is impossible to make a positive statement, but certain inferences can be drawn with some degree of confidence from the data available. If the table of measurements on p. 84, which represents a random sample, is examined it will be found that the numbers of male individuals of different carapace length give, if plotted as a graph, a markedly trimodal curve, with peaks at 8.5, 11.5, and 14.5 mm. The material was obtained between March and August. At the time when the measurements were taken I was not particularly interested in or concerned with the length of life of the animals, for the investigation of which somewhat different methods would have been needed, but it appears legitimate to conclude that the three peaks in the curve indicate that the material included three age-groups, consisting of animals presumably one, two, and three years old. It is worthy of remark that the corresponding curve for females does not show these three definite peaks. This must indicate some difference in the growth of males and females, but more than one form of explanation would fit the facts. It would be idle to speculate on this point, for as already emphasized the data are inadequate, not having been taken with this question in view. It must suffice to say that the data available seem to warrant the conclusion that the time taken to reach maximum size is about three years, though how long the animal may live after growth ceases is left in doubt.

The above conclusion, which, taken alone, might seem to rest on a rather insecure basis, is indirectly supported by the fact that a careful analysis by Runnström (1925) of specially taken measurements on a series of *Calocaris* indicates a life-span of the same order for this fairly closely allied form, namely, 3-4 years, the limit of growth in this case being attained at $3\frac{1}{2}$ years. It appears not improbable that a length of life of the order of three or four years is a usual one amongst the smaller *Macrura* and *Anomura*, though large forms like the Lobster live much longer. The female of *Palaemon Fabricii*, according to Mortensen (1897), lives about three years and the male less.

Whether there is any such sexual difference in *Upogebia* cannot at present be stated.

We may conclude, at any rate, that the duration of life of *Upogebia* is not less than three years, and the evidence, so far as it goes, suggests that the life of the parasite is probably not markedly, if at all, shorter than that of the host. I have come across one or two instances only where the evidence appears indicative of the *Upogebia* having become freed from the parasite in the course of nature (p. 59), and even in these cases it is impossible to say with certainty whether this was accidental or due to the *Gyge* having completed its normal span of life. As the occurrence appears to be so infrequent it seems most probable that it is due to accident, and that the life of host and parasite are normally coextensive. It may be added that if one may judge by the absence of any noticeably deleterious effect on the general vitality and viability of the host, the latter's life is probably not appreciably curtailed as a result of parasitization.

IV. THE EFFECTS OF THE PARASITE ON THE HOST.

A. General Effects.

The influence of the parasite on the general vitality of the host seems to be negligible. There is no noticeable effect on the activity of the animals or on their viability in aquaria. As shown in a later section there is a marked influence on the size and mode of growth of the chela, which is different in the two sexes, but the presence of the *Epicarid* does not appear to prevent the host from reaching as large a size as non-parasitized specimens. On the contrary the largest male I have examined, of 19 mm. carapace length, was a parasitized animal. Moulting is not prevented, as it is in some other cases of parasitization in Crustacea. That such is the case might indeed be safely assumed even in the absence of direct evidence, since it is a necessary corollary of the fact that although parasitization ordinarily takes place when the animals have a carapace length of under 6 mm. parasitized individuals of adult size are no less frequent than young ones. It is thus obvious that a large amount

of growth can and does take place notwithstanding the presence of the parasite. It is nevertheless possible that the rate of growth and the frequency of moults may be lowered as a consequence of parasitization. On this question I have no direct evidence owing to the difficulty of reproducing natural conditions in a laboratory aquarium.

B. Effects on the Sexual Organization.

1. External Characters.

The sexes of *Upogebia littoralis* reach approximately the same size, individuals having a carapace length of about 14–15 mm. being the most numerous. A very few of both sexes reach 18 mm. carapace length. The only two unparasitized individuals of 19 mm. which I have examined happen to have been both females, but observations on a large series show clearly that there is no significant sex difference in size.

The genital pores, as in other Decapods, are on the coxopodites of the second pair of walking legs (sixth thoracic segment) in the female and on those of the fourth and last pair (eighth thoracic segment) in the male. The oviducal pores are conspicuous, showing as translucent-looking oval areas sharply defined from the surrounding, opaque white chitin. The openings of the vasa deferentia are smaller and less easy to see. They are situated nearer the distal ends of the coxopodites than are the female apertures.

I can find no evidence that the genital pores are ever obliterated, however completely the internal reproductive organs are extirpated. In the parasitized female they appear to remain always perfectly distinct, although, as is shown below, complete suppression of the ovary is the normal rule in these animals. In the male it is less easy to be certain, since the pores are at best of times inconspicuous; but, so far as I have been able to ascertain, the little apertures in the chitin always persist even in the most highly modified specimens.

There are two further external differences between the sexes coming under the general designation of secondary sexual characters:

1. The size and mode of growth of the Chelae.—
The chela of the male is relatively large and wide; that of the female is smaller and markedly more slender. The chelae of right and left sides are of the same size.
2. The appendages of the first abdominal segment, which are present in the female, but absent in the male.

The growth and behaviour of these two sets of appendages require detailed consideration.

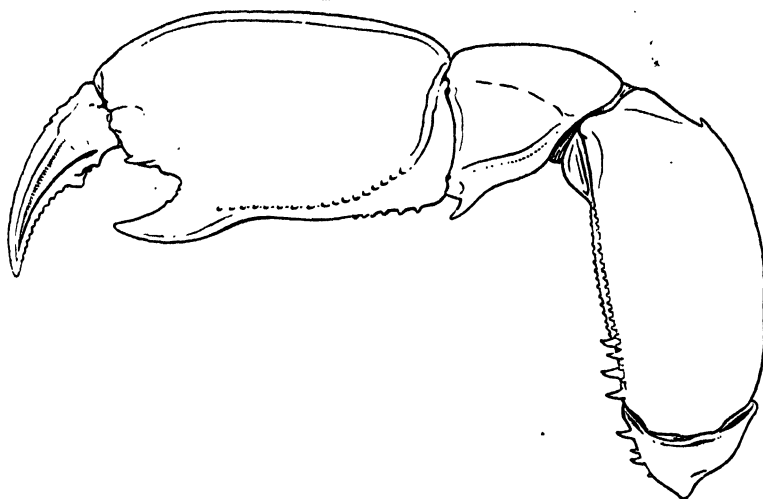
(a) The Structure and Mode of Growth of the Chelae in Normal and Parasitized Animals.

[I wish to repeat here the acknowledgement made on p. 3 to Mr. E. B. Ford for much valuable assistance and advice in connexion with the preparation of the present section. I am deeply indebted to him not merely for advice as to methods, but also for his guidance in matters of interpretation where an unmathematical mind might easily go astray, but I accept full responsibility for the conclusions which I have eventually drawn.]

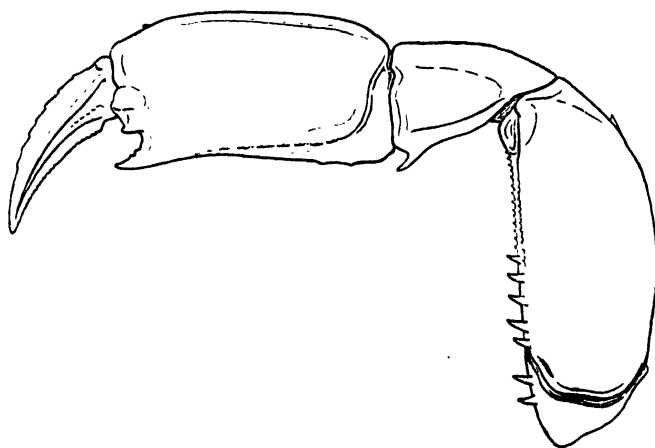
(i) Introductory.

In *Upogebia*, as in many other Decapods, there is a marked secondary sexual difference in the size of the chelae. In the male the chelae are relatively large and stout, with the denticulations of the dactylus more pronounced than in the female and the fixed claw longer, stouter, more curved and more prominent, by which is meant that it stands out so as to form a more distinct angle with the general line of the lower margin of the propodite (Text-fig. 1 A). The female chelae are smaller and markedly more slender. The fixed claw is always much shorter and slenderer than in the male, though its precise length relative to the rest of the chela varies a little (Text-fig. 1, B and C). Up to somewhere about 6 mm. carapace length the chelae are slender and do not differ perceptibly in the two sexes, and judging from actual measurements of a rather small series the average lengths and breadths of the appendages in males and females appear to be in fact substantially the same up to about this size. Miss Gladys Webb (1919) finds that in

TEXT-FIG. 1 A.



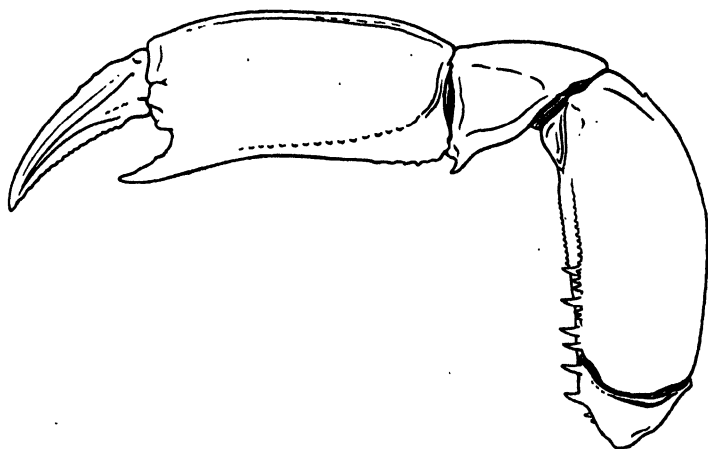
TEXT-FIG. 1 B.



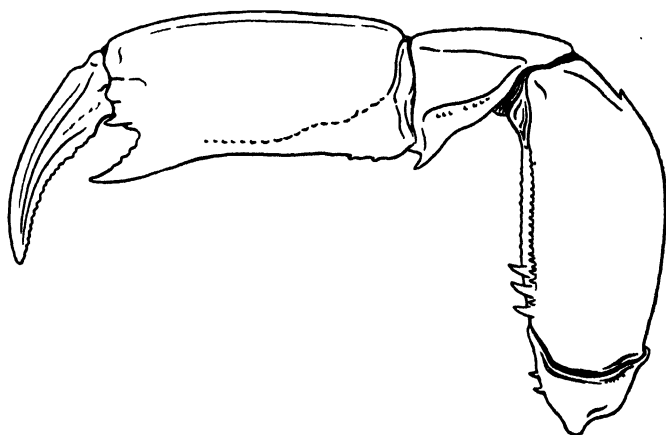
Text figures 1 A-D:

Outlines of chelae of normal and parasitized individuals of *Upogebia littoralis*, to show sexual dimorphism and feminization of the chela of the parasitized male. All the figures are drawn from adult individuals of approximately the same size. *A*, normal male; *B* and *C*, normal female, showing slight variation in the size of the fixed claw of the propodite; *D*, typical parasitized male. (All much enlarged.)

TEXT-FIG. 1C.



TEXT-FIG. 1D.



Upogebia deltura and *stellata* the young animals from the beginning of post-larval life show differences in the form of the chelae corresponding to those which distinguish the adult male and female,¹ and these two types appear to be derived

¹ Her 'class A', which is held to be the female, has a longer and slenderer dactylus than 'class B' (presumed male) and the fixed finger springs from the sixth joint slightly behind the tip.

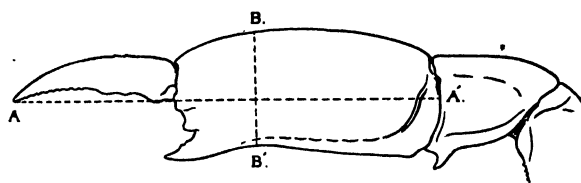
from recognizably distinct types of larvae, of which the one presumed to be the female has apparently one more larval stage than the other. In *Upogebia littoralis*, however, the external differentiation seems to set in later, for after not only examining the chelae of young males and females, but preparing careful camera lucida tracings of a number of them, I find no constant difference in form or proportions between the two sexes, any more than there is in actual length and breadth.¹ From about 6 mm. carapace length onwards, however, a divergence gradually becomes obvious. The chelae of the male grow more rapidly than the rest of the male's body as indexed by the increase in length of the carapace: that is, the male chela shows marked positive heterogony. In parasitized males, on the other hand, the chelae remain small and slender (Text-fig. 1 D) and resemble those of normal females. The feminization of the form of the chela is practically perfect. The fixed finger tends to be a little longer than the average for normal females of corresponding size, but can as a rule be matched almost perfectly by a good many normal female examples. The chelae of parasitized females are not perceptibly altered.

So much is apparent from a quite cursory inspection. The growth of the chelae in normal and parasitized animals was studied more precisely by careful measurements of a considerable series of specimens. Text-fig. 2 shows the method of measurement. Chela length was measured with the dactylus fully extended and chela breadth was taken at the base of the fixed 'finger' of the propodite. The data thus obtained are given in the tables at the end of the present section. Tables I-IV

¹ It is of course necessary to examine a sufficient series to allow for individual variation. After examining two or three specimens I gained the impression that even in these very small animals, of 3 or 4 mm. carapace length, the fixed finger in the male was slightly more prominent and more curved than in the female. When further material was examined, however, it quickly became clear that this condition was merely individual, not constant in all males and, on the other hand, paralleled exactly in some of the females. It may be mentioned that in general the fixed finger in very young females tends to be relatively slightly larger and better developed than in the adults.

are correlation tables showing the range of chela length found in individuals of given carapace length; V-VIII are similar tables for chela breadth. The chela measurements were recorded to the nearest 0.25 of a millimetre, but for the purpose of the correlation tables it was found sufficient to reckon lengths to the nearest millimetre and breadths to the nearest half millimetre. The original measurements taken at Naples included animals of about 8 mm. carapace length upwards, but when the results were worked out it was found desirable to carry back the measurements rather farther than this. This was

TEXT-FIG. 2.



Outline of normal female chela to show method of measurement.
A-A', chela length; B-B', chela breadth.

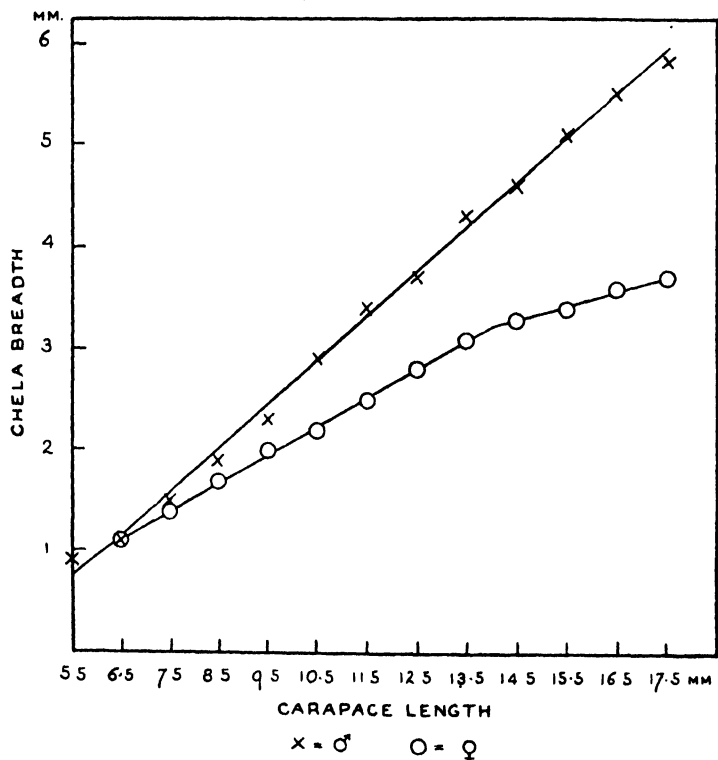
done with a small series of specimens of about 5-7 mm. carapace length which remained from material preserved for sectioning, amongst which, however, there were no parasitized females left. The chelae of these small animals were measured to the nearest 0.1 mm. by means of a microscope, camera lucida, and squared paper. They do not appear in the correlation tables, in which, as just stated, the measurements, being for larger animals, are reckoned a little less precisely, but the mean measurements for these smaller sizes and the number measured are recorded in Tables IX-XII, together with the corresponding particulars for the older animals.

Figures in brackets in the correlation tables indicate rare cases where the chela measurements are more or less strikingly outside the normal range for the body-size in question. These are regarded as individual abnormalities and are left out of account in calculating the average chela measurements of the group. The total number measured was: Normal males, 268;

normal females, 219; parasitized males, 109; parasitized females, 74.

The mode of growth of the chelae in normal and parasitized *Upogebia* is best illustrated by graphs constructed from the data given in the tables. In order to obtain a clear general idea

TEXT-FIG. 3.



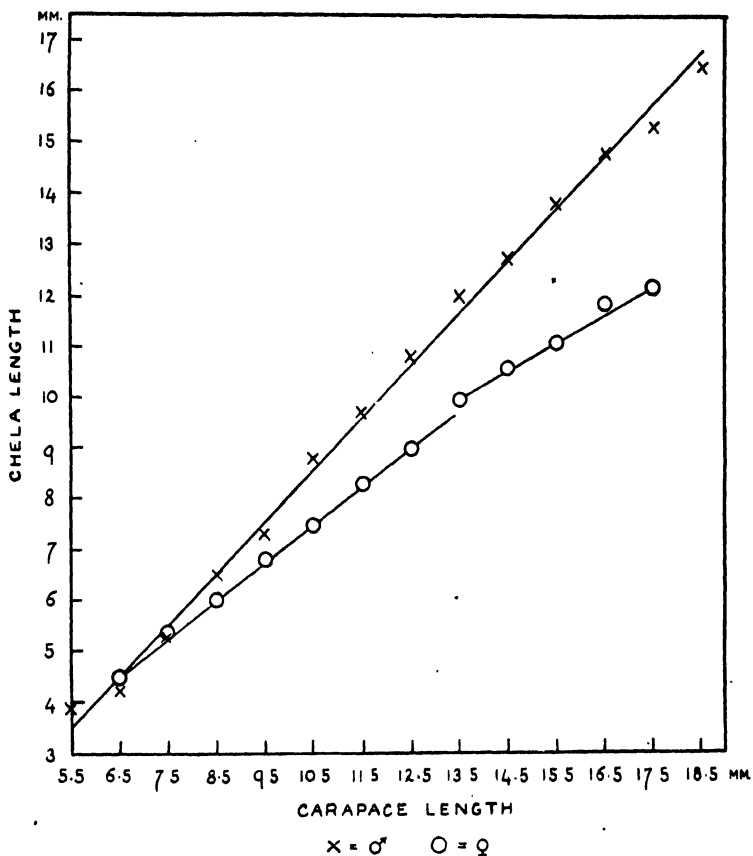
Chela breadth in normal male and female plotted against carapace length.

of what is happening and to compare the conditions in normal and parasitized material of both sexes, graphs constructed from the direct measurements without any manipulation are the most satisfactory and trustworthy, and these will now be discussed.

(ii) Absolute increase in size of the Chelae.
Chela breadth and length.

(a) Normal male and normal female (Text-figs. 3

TEXT-FIG. 4.

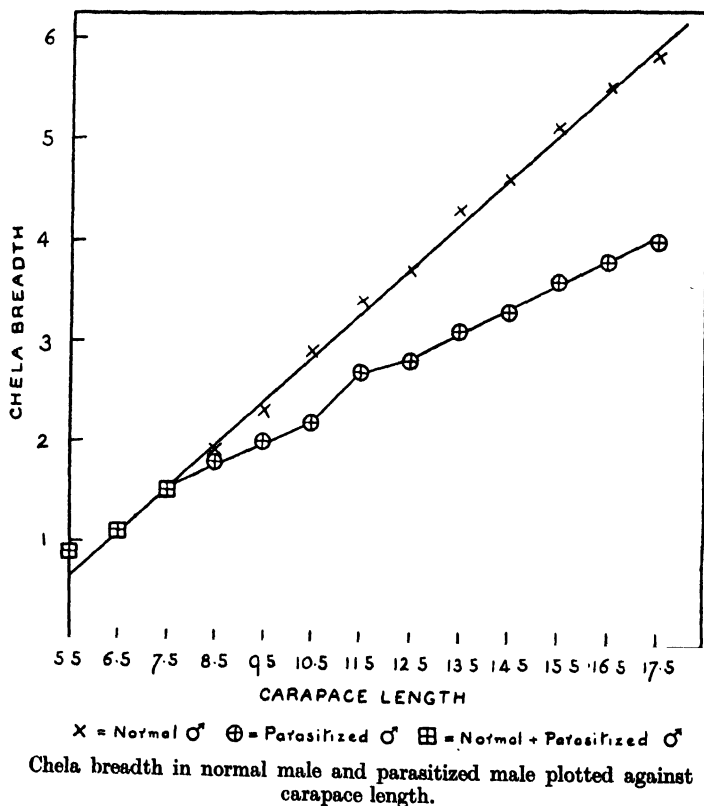


Chela length in normal male and female plotted against carapace length.

and 4).—The rate of growth of the male chela is shown to be absolutely constant, for the graph is a straight line. In the female there is a slight change in the slope of the line in the

region of 13 mm. carapace length. It is natural to connect this with sexual maturity, such a change in the growth-rate at this period being a well-known phenomenon. As a matter of fact

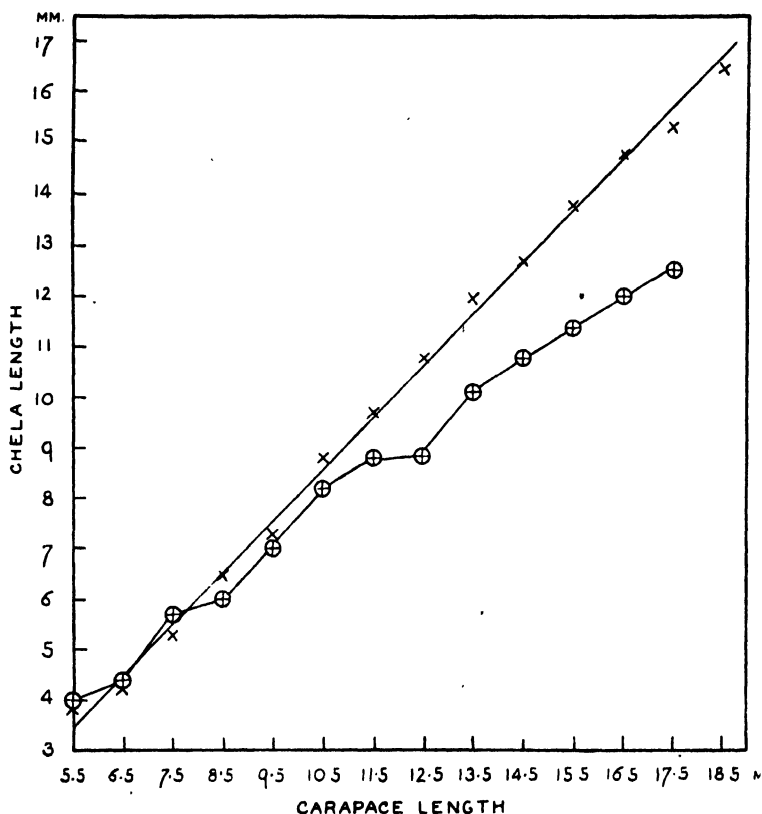
TEXT-FIG. 5.



female *Upogebia* become sexually-mature considerably before this size, individuals of as little as 8 mm. carapace length being found with eggs, but it is possible that the majority do not lay much before 12 mm. carapace length or that there is a rather considerable lag between the laying of the first batch of eggs and the time when the resultant drain on the resources

of the organism begins to be reflected in the growth-rate. Or possibly the explanation lies in a combination of these two suggestions.

TEXT-FIG. 6.



X = Normal ♂ ⊕ = Parasitized ♂

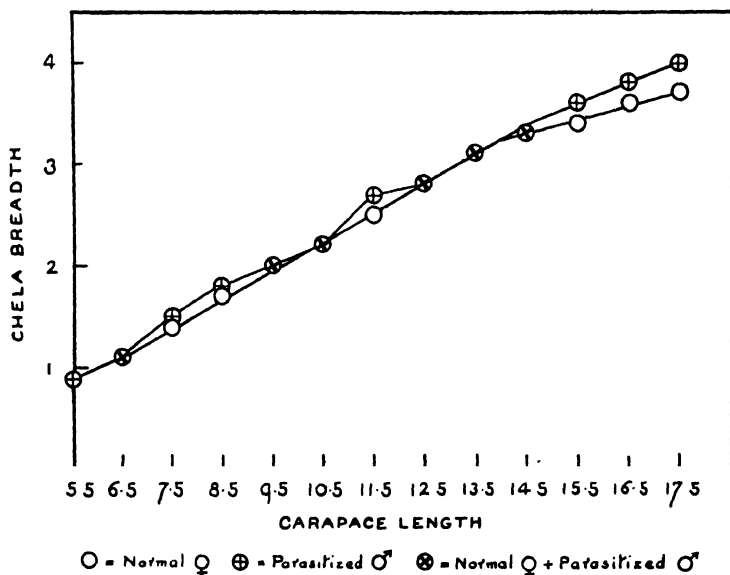
Chela length in normal male and parasitized male plotted against carapace length.

(b) Normal male and parasitized male (Text-figs. 5 and 6).—The chela of the parasitized male grows more slowly than in the normal male. The bend in the course of the parasitized male line in Text-fig. 6 will be referred to again. The

small irregularity at 11.5 mm. in Text-fig. 5 is probably fortuitous. Such irregularities are not unexpected in parasitized animals, in which the measurements are likely to vary more than in normals owing to slight differences in the age at parasitization, individual susceptibility, &c.

(c) Normal female and parasitized male (Text-

TEXT-FIG. 7.



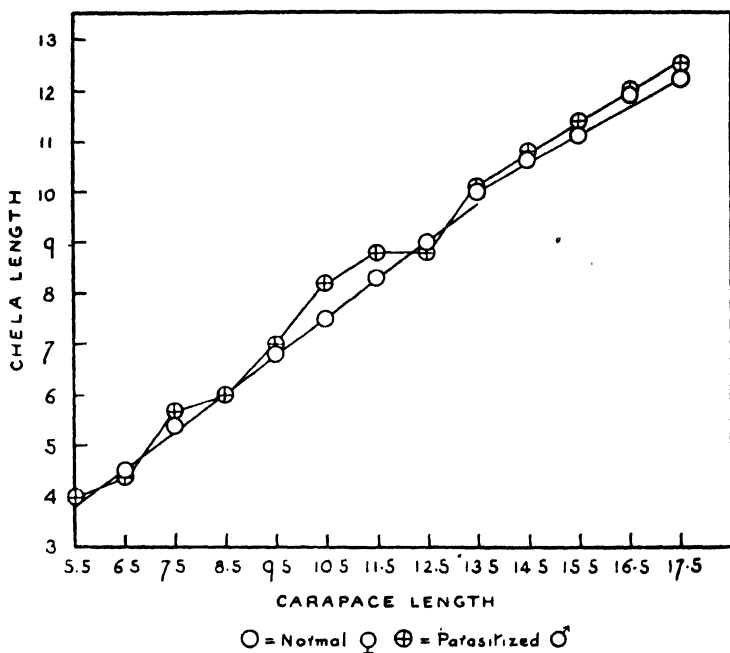
Chela breadth in normal female and parasitized male plotted against carapace length.

figs. 7 and 8).—When the graph for the parasitized male is superimposed on that for the normal female they are found to coincide extremely closely. The figures thus show graphically that there is a definite feminization of the male. It should be observed, however, that in the case of breadth three consecutive points for the parasitized male are distinctly above the corresponding part of the female line. The differences are not large, but are believed not to be fortuitous. Their probable significance is made more clear by the graphs illustrating the relative

growth of the chelae as compared with the carapace and will be considered further in the section dealing with this.

(d) Normal female and parasitized female (Text-figs. 9 and 10).—Parasitization scarcely affects the female

TEXT-FIG. 8.



Chela length in normal female and parasitized male plotted against carapace length.

chelae. They tend to average a very little smaller than those of normal females, but the difference is altogether trivial.

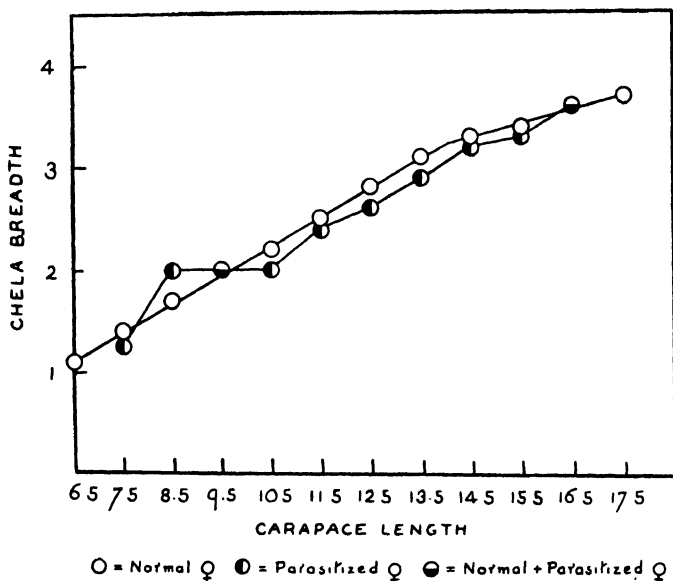
(iii) Relative Growth of the Chelae.

In order to bring out the changes in the relative proportions of the chelae as compared with the carapace length, the method employed by Huxley (1924*a*) and others is valuable, namely, to plot the ratio chela length (or breadth) : carapace length as a percentage against carapace length. If the chela is growing

relatively faster than the body the graph is a line sloping upwards, if it is increasing relatively more slowly than the rest of the body the line slopes downwards, while if there is no relative change, but the size of the appendage relatively to the rest of the body remains constant, the line is horizontal.

This method has its limitations inasmuch as all experimental

TEXT-FIG. 9.



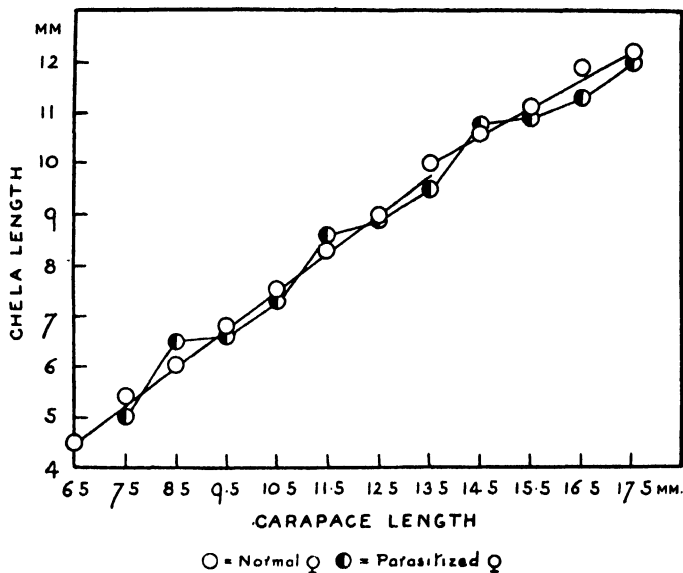
Chela breadth in normal female and parasitized female plotted against carapace length.

errors and trivial fluctuations are greatly exaggerated, and it is important to avoid being led astray by these. The graphs based on the direct measurements should be used as a check.

The ratios $\frac{\text{Chela length (or breadth)} \times 100}{\text{Carapace length}}$ for every millimetre carapace length are given in Tables IX–XII. When these figures were plotted the curves for normal male and female were found to be only mildly irregular, but those for parasitized animals were much more considerably so, especially

where the numbers were small. Irregularities of this kind can be smoothed out to a considerable extent by combining the groups, two or more at a time, and calculating the average chela length or breadth for the combined classes; but the drawback

TEXT-FIG. 10.

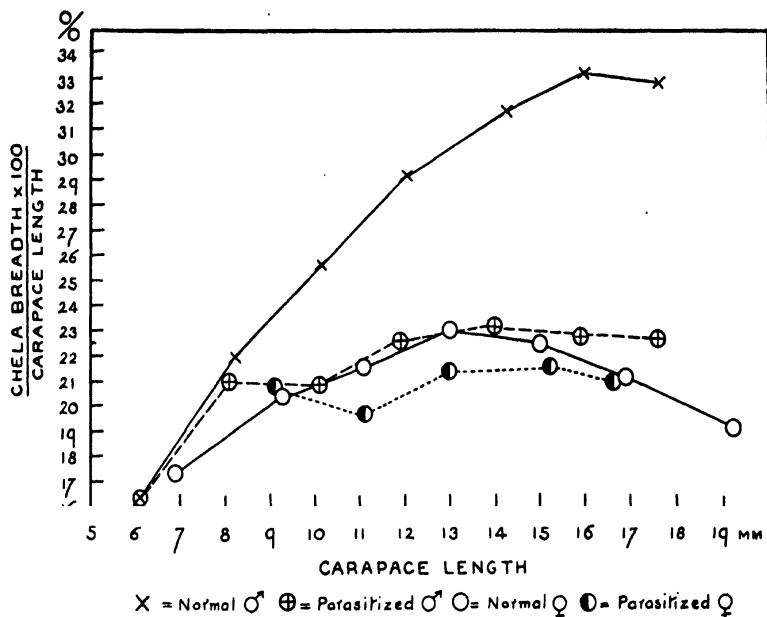


Chela length in normal female and parasitized female plotted against carapace length.

of this proceeding is that if the groups are too large the smoothing may go too far, levelling out real differences in the figures and giving an appearance of uniformity which may be quite misleading. A judicious regrouping into classes in which the range of size is still quite small is, however, hardly open to this objection. In the present case the original classes of 1 mm. carapace length each were combined into classes of 2 mm. each and the average chela measurements and required ratios recalculated for these, the result appearing in Tables XIII-XVI. It is believed that the graphs so obtained convey, in conjunction with those already considered, an altogether more useful and readily intelligible picture of what is going on than those based

on the 1 mm. groups (not figured), in which non-significant irregularities considerably obscure the issue, but which lead on closer study to just the same conclusions.

TEXT-FIG. 11.



Chela breadth in normal and parasitized males and females, expressed as percentages of the carapace length, plotted against carapace length: to show relative growth in breadth of the chelae.

Chela breadth (Text-fig. 11).

(a) Normal male and female.—Reference to Text-fig. 11 shows that the male chela—as indeed is obvious from a mere inspection of specimens—is growing in breadth relatively much faster than the rest of the body, but the relative rate of growth shows a progressive slight decrease until when the animal is nearing the maximum size it slows down to approximately the same rate as that of the carapace. The original measurements for the 1 mm. groups (Table IX) show that the peak of the curve comes at 16.5 mm. carapace length. The figure for 17.5 mm. is

only less by an insignificant amount, while that for 18.5 mm. is a little less again, but is based on two specimens only. In the circumstances it is difficult to say whether there is a real though slight falling off in the size of the chela relatively to the carapace after 16.5 mm. or whether the chela should rather be regarded as just keeping pace with the carapace after this point, the small relative decrease which the figures suggest not being significant. For present purposes, however, the point is not of much importance. The point of divergence of the male and female curves is evidently at about 6-7 mm. carapace length. The graph shows that the female chela, though growing less rapidly than that of the male, is nevertheless during the earlier part of life growing quite considerably faster than the rest of the body, a fact not obvious from an examination of the animals. The maximum relative breadth of the female chela is reached at about 13 mm. carapace length, and after this there is a relative decrease. There is positive heterogony of the appendages up to about 13 mm. carapace length and negative heterogony after. This is hinted at by the slight change in the slope of the line when the direct measurements of chela breadth are plotted against carapace length (Text-fig. 3), but by the present method it is rendered obvious.

(b) Parasitized male and female.—The chela of the parasitized male after growing for a little while at the same or practically the same rate as in the normal male is then slowed down to essentially the same rate as the female. Up to about 14 mm. carapace length the agreement is exceedingly close, but from this point onwards the two curves follow a slightly different course. The difference is not large and it might be doubted whether it is significant were it not for the fact that it is sufficient to be quite clearly recognizable even in the graph of the direct measurements. It thus seems reasonable to conclude that the growth-rate of the parasitized male chela, having been brought down to the female level, tends not to decrease quite as markedly after 13 mm. carapace length as in the normal female, but remains more nearly constant relatively to the carapace. It may be suggested that this small difference is due to the fact that the real female has to support an actively functioning

ovary while the feminized male has not this drain on its resources, though it ought perhaps to be added in this connexion that the corresponding curves for chela length do not show a comparable divergence. In the case of the parasitized female, figures for the smaller sizes are not available, and the total number measured is smaller than in the other classes. The fact that the absolute breadth of the chela in parasitized females averages slightly but consistently less than in normal animals is here emphasized. It is thought probable that the drop at about 11 mm. is exaggerated, and if this is so the curve, though close to that of the normal female, has a less definite peak than in the unparasitized animal, its general trend being more nearly horizontal. This would suggest that in the parasitized female the disharmony between the rates of growth of chela and carapace is lessened and the appendage tends to grow at more nearly the same rate as the rest of the body. If this supposition is correct it would be consistent with the suggestion made above that the relative decrease in the breadth of the chela in the later phases of growth of the normal female is directly connected with the demands made on the organism by the ovary.

Chela length (Text-fig. 12).

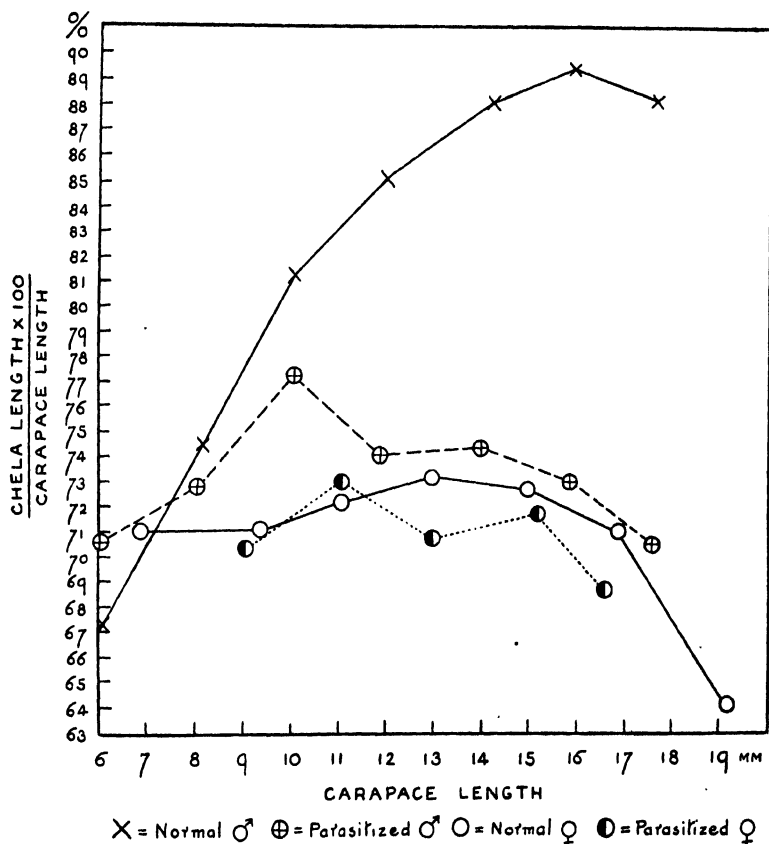
(a) Normal male and female.—The normal male curve is quite similar to that for breadth with again a slight drop at the end. The remarks made in the case of chela breadth apply in much the same way here, *mutatis mutandis*.

In the normal female the curve, like that for breadth, reaches its apex at 13 mm. carapace length, but the positive heterogony of the first phase is less definite, the growth-rate in respect of length being evidently more nearly uniform with that of the rest of the body. After 13 mm. there is a gradual, but eventually considerable, falling off—i.e. there is a distinct negative heterogony.

(b) Parasitized male and female.—The curves, for parasitized animals based on the 1 mm. groups show more conspicuous irregularities than in the case of breadth, suggesting that there is rather greater variability in the effects of parasitization on the length than on the breadth of the chela. Reference

to Tables IX and XI will show that amongst the youngest animals the mean chela lengths for parasitized males tend even

TEXT-FIG. 12.



Chela length in normal and parasitized males and females, expressed as percentages of the carapace length, plotted against carapace length : to show relative increase in length of the chelae.

to exceed, in the small series examined, the corresponding averages for normal males. As a result the first point on the parasitized male curve in Text-fig. 12 is actually above that of the normal male. It may be concluded that up to 7 or 8 mm.

carapace length the parasite exerts no appreciable effect. The behaviour after this is not quite the same as in the case of breadth, where the growth-rate shows a definite slowing down at about 8 mm. carapace length and by 10 mm. is substantially the same as in the female. The length of the chela is not so quickly influenced. Growth in length continues evidently only a little less rapidly than in the uninfected male up to about 10-10.5 mm. carapace length, so that even in the graph constructed from the direct measurements the corresponding part of the parasitized male line is, as has been already noticed, quite distinctly above that of the female. After this it begins to be pulled down. By about 12 mm. it has been depressed to practically the female level, and thereafter the curve follows that of the normal female very closely at a level of mean absolute size only higher than that of the female by an almost negligible amount. The small divergence between the parasitized male and normal female curves in the latter part of life which is indicated in the case of chela breadth is not observed in the curves for length.

In the case of the parasitized female it has already been mentioned that the series measured was rather small and did not extend back to quite such small sizes as the other classes. It is improbable that any significance is to be attached to the precise form of the curve obtained. It seems sufficient to say that it fluctuates close to the normal female curve and for the most part a little below it.

(iv) Conclusions.

It may be convenient to round off the present section by reviewing the main conclusions arrived at, which are as follows :

The growth-rate of the male chela in respect of both length and breadth is markedly greater than that of the body, as indexed by the carapace length, until the animal is nearing the maximum size, when it decreases to approximately the same rate as the rest of the body or even slightly slower.

The female chela also grows more rapidly than the body, though less strikingly so than in the male, up to 13 mm. carapace length, after which the growth-rate becomes less than that

of the body. The positive heterogony of the first phase is marked in respect of breadth, but only slight in respect of length.

The chela of the parasitized male undergoes a definite feminization in its growth and proportions, the only significant difference, once agreement has been reached, being that after 13 mm. carapace length it tends to maintain a rate of growth in breadth approximately uniform with that of the rest of the body, rather than to undergo a definite relative decrease, as in the normal female. It is suggested that this difference is due to the fact that the parasitized male is not subject to the drain on its resources caused by egg-production in the female.

Feminization occurs distinctly later in respect of length than of breadth. It is found that growth in breadth proceeds at the same or about the same rate as in the normal male up to about 7 or 8 mm. carapace length, when a slowing down occurs, resulting in substantial agreement with the female being reached at about 10 mm. carapace length. Growth in length proceeds at only a slightly less rapid average rate than in the normal male up to about 10 mm. carapace length, when it enters a phase of relative decrease ending in the attainment of substantial agreement with the female at about 12 mm. carapace length.

The growth of the chela in the parasitized female is only very slightly affected by parasitization, the average measurements tending to be merely a trifle smaller than in normal females.

(v) Appendix: Tables of Measurements.

TABLES I-IV.

CORRELATION TABLES—CHELA LENGTH AND CARAPACE LENGTH

TABLE I.

NORMAL MALE—CHELA LENGTH.

Carapace length in millimetres.

<i>Chela length in mm.</i>	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	<i>Totals.</i>
5	1	1
6	9	1	10
7	8	7	1	16
8	1	5	4	2	12
9	11	8	1	20
10	2	21	6	29
11	3	19	6	4	32
12	3	10	10	2	25
13	5	24	10	1	40
14	3	22	5	1	..	31
15	1	5	17	5	..	28
16	1	2	1	4
17	1	1	2
Totals	19	13	18	34	29	21	42	39	24	9	2	250

TABLE II.

NORMAL FEMALE—CHELA LENGTH.

Carapace length in millimetres.

<i>Chela length in mm.</i>	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	<i>Totals.</i>
6	3	3	6
7	..	9	9	18
8	9	15	2	26
9	7	20	5	1	33
10	3	18	14	4	39
11	(1)	..	6	19	27	1	1	55
12	2	7	7	4	1	1	22
13	2	..	1	3
Totals	3	12	18	23	25	29	36	38	8	7	1	2	202

TABLE III.

PARASITIZED MALE—CHELA LENGTH.

Carapace length in millimetres.

<i>Chela length in mm.</i>	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	<i>Totals.</i>
6	6	6
7	..	3	1	4
8	4	4	1	(1)	10
9	1	9	8	3	21
10	1	1	7	6	1	16
11	..	(1)	4	4	7	..	1	17
12	3	4	7	2	..	1	17
13	(1)	1	..	5	7
Totals	6	4	5	15	11	14	13	13	8	8	0	1	98

TABLE IV.

PARASITIZED FEMALE—CHELA LENGTH.

Carapace length in millimetres.

<i>Chela length in mm.</i>	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.6	17.5	<i>Totals.</i>
6	1	2	3
7	1	3	4	8
8	2	4	2	1	9
9	5	8	6	19
10	1	6	1	3	11
11	1	4	9	5	..	19
12	(1)	1	2	1	5
Totals	2	5	7	9	11	14	5	13	7	1	74

TABLES V-VIII.

CORRELATION TABLES—CHELA BREADTH AND CARAPACE LENGTH

TABLE V.

NORMAL MALE—CHELA BREADTH.

Carapace length in millimetres.

<i>Chela breadth in mm.</i>	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	<i>Totals.</i>
1.5	3	3
2.0	16	7	2	25
2.5	..	4	1	1	6
3.0	..	2	12	10	3	27
3.5	3	17	11	..	2	33
4.0	6	15	12	4	37
4.5	5	21	4	30
5.0	4	12	27	7	2	..	52
5.5	3	8	9	20
6.0	7	6	2	15
6.5	1	1	..	2
Totals	19	13	18	34	29	21	42	39	24	9	2	250

TABLE VI.

NORMAL FEMALE—CHELA BREADTH.

Carapace length in millimetres.

<i>Chela breadth in mm.</i>	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	<i>Totals.</i>
1.5	2	1	3
2.0	1	11	11	4	1	28
2.5	7	14	9	1	1	32
3.0	6	16	24	14	9	1	1	71
3.5	6	22	27	4	2	1	1	63
4.0	(1)	2	3	4	..	1	11
4.5	(1)	1
Totals	3	12	18	25	26	32	37	38	8	7	1	2	209

TABLE VII.
PARASITIZED MALE—CHELA BREADTH.

Carapace length in millimetres.

<i>Chela breadth in mm.</i>	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	<i>Totals.</i>
1.5	2	(1)	3
2.0	4	3	4	1	12
2.5	6	4	3	1	14
3.0	..	(1)	1	8	6	5	5	3	29
3.5	6	5	5	5	1	22
4.0	2	5	2	7	16
4.5	1	1	2
Totals	6	4	5	15	11	14	13	13	8	9	98

TABLE VIII.
PARASITIZED FEMALE—CHELA BREADTH.

Carapace length in millimetres.

<i>Chela breadth in mm.</i>	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	<i>Totals.</i>
2.0	2	5	7	1	1	16
2.5	8	7	4	19
3.0	3	9	3	6	..	1	22
3.5	1	2	7	5	..	15
4.0	2	..	2
Totals	2	5	7	9	11	14	5	13	7	1	74

TABLES IX-XII.

MEAN ABSOLUTE MEASUREMENTS OF CHELAE AND THE SAME
MEASUREMENTS RECKONED AS PERCENTAGES OF THE
CARAPACE LENGTH

TABLE IX.—NORMAL MALE.

<i>Carapace length in mm.</i>	<i>Mean chela length.</i>	<i>No. mea- sured.</i>	<i>Chela length × 100</i>	<i>Mean chela breadth.</i>	<i>No. mea- sured.</i>	<i>Chela breadth × 100</i>
			<i>Carapace length.</i>			<i>Carapace length.</i>
5.5	3.9	3	70.9	0.9	3	16.4
6.5	4.2	4	64.6	1.1	5	16.9
7.5	5.3	10	70.7	1.5	10	20.0
8.5	6.5	19	76.5	1.9	19	22.4
9.5	7.3	13	76.8	2.3	13	24.2
10.5	8.8	18	83.8	2.9	18	27.6
11.5	9.7	34	84.4	3.4	34	29.6
12.5	10.8	29	86.4	3.7	29	29.6
13.5	12.0	21	88.9	4.3	21	31.9
14.5	12.7	42	87.5	4.6	42	31.7
15.5	13.8	39	89.0	5.1	39	32.9
16.5	14.8	24	89.7	5.5	24	33.3
17.5	15.3	9	87.5	5.8	9	33.1
18.5	16.5	2	89.1	6.0	2	32.4

TABLE X.—NORMAL FEMALE.

<i>Carapace length in mm.</i>	<i>Mean chela length.</i>	<i>No. mea- sured.</i>	<i>Chela length × 100</i>	<i>Mean chela breadth.</i>	<i>No. mea- sured.</i>	<i>Chela breadth × 100</i>
			<i>Carapace length.</i>			<i>Carapace length.</i>
6.5	4.5	6	69.2	1.1	6	16.9
7.5	5.4	4	72.0	1.4	3	18.7
8.5	6.0	3	70.6	1.7	3	20.0
9.5	6.8	12	71.6	2.0	12	21.1
10.5	7.5	18	71.4	2.2	18	21.0
11.5	8.3	22	72.2	2.5	24	21.7
12.5	9.0	25	72.0	2.8	26	22.4
13.5	10.0	29	74.1	3.1	31	23.0
14.5	10.6	36	73.1	3.3	37	22.8
15.5	11.1	38	71.6	3.4	38	21.9
16.5	11.9	8	72.1	3.6	8	21.8
17.5	12.2	7	69.7	3.7	7	21.1
18.5	12.0	1	64.9	3.5	1	18.9
19.5	12.5	2	64.1	3.8	2	19.5

TABLE XI.—PARASITIZED MALE.

<i>Carapace length in mm.</i>	<i>Mean chela length.</i>	<i>No. measured.</i>	<i>Chela length × 100</i>	<i>Mean chela breadth.</i>	<i>No. measured.</i>	<i>Chela breadth × 100</i>
			<i>Carapace length.</i>			<i>Carapace length.</i>
5.5	4.0	3	72.7	0.9	3	16.4
6.5	4.4	5	67.7	1.1	5	16.9
7.5	5.7	3	76.0	1.5	3	20.0
8.5	6.0	6	70.5	1.8	6	21.2
9.5	7.0	3	73.7	2.0	3	21.1
10.5	8.2	5	78.1	2.2	5	21.0
11.5	8.8	14	76.5	2.7	15	23.5
12.5	8.8	11	70.4	2.8	10	22.4
13.5	10.1	14	74.8	3.1	14	23.0
14.5	10.8	13	74.5	3.3	13	22.8
15.5	11.4	13	73.5	3.6	13	23.2
16.5	12.0	7	72.7	3.8	7	23.0
17.5	12.5	8	71.4	4.0	9	22.9
18.5	12.0	1	64.9

TABLE XII.—PARASITIZED FEMALE.

<i>Carapace length in mm.</i>	<i>Mean chela length.</i>	<i>No. measured.</i>	<i>Chela length × 100</i>	<i>Mean chela breadth.</i>	<i>No. measured.</i>	<i>Chela breadth × 100</i>
			<i>Carapace length.</i>			<i>Carapace length.</i>
7.5	5.0	1	66.7	1.5	1	20.0
8.5	6.5	2	76.5	2.0	2	23.5
9.5	6.6	5	69.5	2.0	5	21.1
10.5	7.3	6	69.5	2.0	7	19.0
11.5	8.6	9	74.8	2.4	9	20.9
12.5	8.9	11	71.2	2.6	11	20.8
13.5	9.5	14	70.4	2.9	14	21.5
14.5	10.8	5	74.4	3.2	5	22.1
15.5	10.9	13	70.3	3.3	13	21.8
16.5	11.3	7	68.5	3.6	7	21.8
17.5	12.0	1	68.6	3.0	1	17.2

TABLES XIII-XVI.

THE CHELA MEASUREMENTS RECALCULATED FOR GROUPS
OF 2 mm. CARAPACE LENGTH

TABLE XIII.—NORMAL MALE.

<i>Carapace length groups.</i>	<i>Mean of group.</i>	<i>Mean chela length for group.</i>	<i>Chela length × 100</i>	<i>Mean chela breadth for group.</i>	<i>Chela breadth × 100</i>
			<i>Carapace length.</i>		<i>Carapace length.</i>
5.5- 6.5	6.1	4.1	67.3	1.0	16.4
7.5- 8.5	8.2	6.1	74.4	1.8	22.0
9.5-10.5	10.1	8.2	81.2	2.6	25.7
11.5-12.5	12.0	10.2	85.0	3.5	29.2
13.5-14.5	14.2	12.5	88.0	4.5	31.7
15.5-16.5	15.9	14.2	89.3	5.3	33.3
17.5-18.5	17.6	15.5	88.1	5.8	32.9

TABLE XIV.—NORMAL FEMALE.

<i>Carapace length groups.</i>	<i>Mean of group.</i>	<i>Mean chela length for group.</i>	<i>Chela length × 100</i>	<i>Mean chela breadth for group.</i>	<i>Chela breadth × 100</i>
			<i>Carapace length.</i>		<i>Carapace length.</i>
6.5- 7.5	6.9	4.9	71.0	1.2	17.4
8.5- 9.5	9.3	6.6	71.0	1.9	20.4
10.5-11.5	11.1	8.0	72.1	2.4	21.6
12.5-13.5	13.0	9.5	73.1	3.0	23.1
14.5-15.5	15.0	10.9	72.7	3.4	22.6
16.5-17.5	16.9	12.0	71.0	3.6	21.3
18.5-19.5	19.2	12.3	64.1	3.7	19.3

TABLE XV.—PARASITIZED MALE.

<i>Carapace length groups.</i>	<i>Mean of group.</i>	<i>Mean chela length for group.</i>	<i>Chela length × 100</i>	<i>Mean chela breadth for group.</i>	<i>Chela breadth × 100</i>
			<i>Carapace length.</i>		<i>Carapace length.</i>
5.5- 6.5	6.1	4.3	70.5	1.0	16.4
7.5- 8.5	8.1	5.9	72.8	1.7	21.0
9.5-10.5	10.1	7.8	77.2	2.1	20.9
11.5-12.5	11.9	8.8	74.0	2.7	22.7
13.5-14.5	14.0	10.4	74.3	3.2	23.3
15.5-16.5	15.9	11.6	73.0	3.7	22.9
17.5-18.5	17.6	12.4	70.5	4.0	22.8

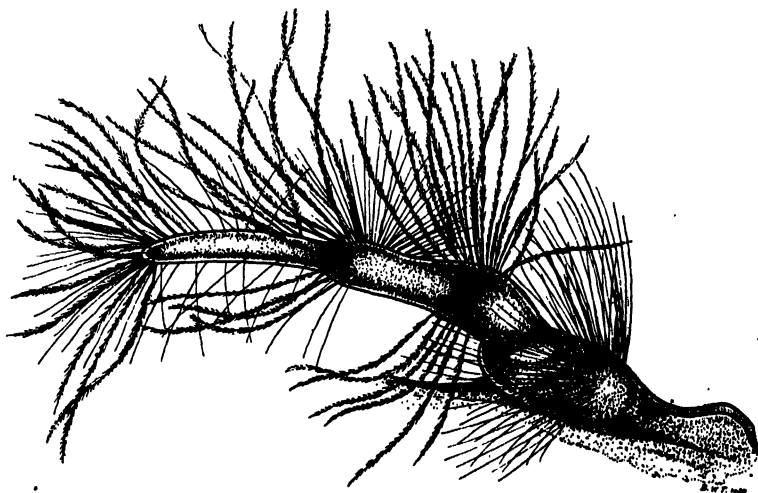
TABLE XVI.—PARASITIZED FEMALE.

<i>Carapace length groups.</i>	<i>Mean of group.</i>	<i>Mean chela length for group.</i>	<i>Chela length × 100</i>	<i>Mean chela breadth for group.</i>	<i>Chela breadth × 100</i>
			<i>Carapace length.</i>		<i>Carapace length.</i>
7.5- 9.5	9.1	6.4	70.3	1.9	20.9
10.5-11.5	11.1	8.1	73.0	2.2	19.8
12.5-13.5	13.0	9.2	70.8	2.8	21.5
14.5-15.5	15.2	10.9	71.7	3.3	21.7
16.5-17.5	16.6	11.4	68.7	3.5	21.1

(b) The Structure and Growth of the First Pair of Abdominal Appendages.

The pleopods of the abdominal segments after the first are similar in the two sexes. The first abdominal segment is without

TEXT-FIG. 13.

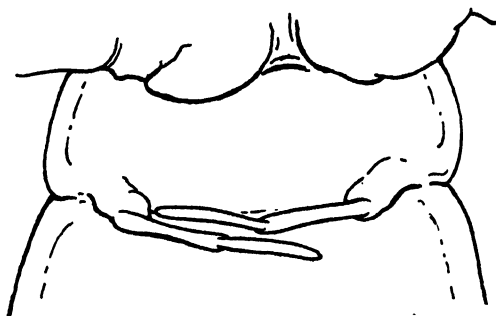


First abdominal appendage (right side) of normal female with plumose setae fully developed (enlarged).

appendages in the male, but possesses them in the female. They are simple, uniramous, two-jointed structures, bearing in the adult long hairs or setae, which are of two types, simple and plumose (Text-fig. 13). The latter are much the longer and

stouter and to them the eggs in the ovigerous female are attached, as well as to the setae of the other pleopods. The setae of the first abdominal appendages are absent in young animals, and the material at my disposal seems to show definitely that they are absent in females of all ages outside the breeding season, and must in fact be regarded as seasonally developed structures characteristic of sexually mature animals which are either bearing eggs or are about to bear or have lately borne them.

TEXT-FIG. 14.



Ventral view of the anterior region of the abdomen of *Upogebia littoralis*, to show the first pair of abdominal appendages in the natural position.

The appendages themselves appear relatively late in life, long after the other pleopods. If an attempt is made, however, to define any sort of series of growth stages, it becomes clear that they exhibit quite considerable variation in the time of their appearance and in the degree of their development at given body sizes. They are not present in any specimen which I have examined of less than 11.5 mm. total length. They make their appearance between this length and 14 mm., usually at about 12 mm. The stage at which, when lying in the normal position turned inward at right angles to the long axis of the body, they just meet at their tips, occurs between total lengths of about 18–23 mm. They are found overlapping one another slightly between 21–3 mm., while the full size, at which the tip of one reaches approximately to the base of the other, is arrived at between about 23 and 33 mm. As 23 mm. total length

corresponds to a carapace length of approximately 9 mm.¹ and a few individuals of 8–9 mm. may be found bearing eggs it may be stated that the completion of growth of the appendage probably coincides roughly with sexual maturity.

The setae do not appear until growth of the appendage is nearly or quite completed, and sometimes not until after this. Their presence is obviously a *sine qua non* for the attachment of the eggs, so that those individuals which breed early in life must also develop the setae earlier, while those which develop them rather later are doubtless those which are less precocious in arriving at sexual maturity. I may add that I have been able to demonstrate such a variation in the time of arrival at sexual maturity directly by microscopical examination of gonads, and I shall return to this point again (pp. 63 and 65).

I have met with no case in the larger parasitized animals where the first pair of abdominal appendages were in the early stages of development. Such stages are only found in quite young specimens, as I have already emphasized in presenting the evidence which convinces me that fixation of the parasite occurs entirely or almost entirely in the young.

If it is desired to institute an exact comparison between the time of appearance and rate of growth of these appendages in normal females and in parasitized females and males a complication is introduced by the considerable variability which, as indicated above, even the normal animals show. Measurements of a series of parasitized females seem to justify the conclusion that the rate of growth of the appendages is slightly but definitely slowed down by the action of the parasite. It also appears that in a certain number of parasitized individuals the appendages may never quite reach the full normal size, or possibly having reached the full size relatively to the body they may subsequently fail quite to keep pace with further bodily growth. I am led to this conclusion by the fact that in a certain

¹ The above observations were made at an early stage of the work and the sizes of the animals were recorded in terms of total length instead of carapace length, the measurement generally employed in this account. It may be noted that roughly 11.5 total length = about 4 mm. carapace length.

number of the larger parasitized individuals, in which it is unlikely that the appendages are still growing, these organs are still a trifle short as compared with normal animals. Some of these specimens have the appendages 'feathered', and it has been seen that the development of the feathery setae is the final stage in the development of the first abdominal appendage. It should be stated, however, that in other examples the said appendages are fully as long as in normal animals and the difference when it exists is only very small.

In parasitized males the range of variation in the degree of development of the first abdominal appendages at given body-sizes is greater than in normal females. This is to be expected, since some will be infected early enough for the appendages to appear at about the same stage as in females, others will not be attacked until after this stage is passed. As stated in a previous section (p. 10), it appears that fixation may take place up to the time when the *Upogebia* is 16-17 mm. long (carapace length 6-6.5 mm.), but probably not normally after this, since parasitized males in which fixation is so recent that the female appendages have not yet begun to appear may be found up to the size named, but not beyond. It must be presumed that after fixation of the parasite an appreciable time will elapse before a sufficient effect is produced on the metabolism of the host to cause (or to permit) the female appendages to begin to develop, but evidently the response is fairly rapid.

At the season when normal individuals are breeding and females have their first abdominal appendages fully feathered, parasitized individuals of both sexes may be found with these setae quite well developed and others in which they are developed only feebly or not at all. It would seem, therefore, that under the parasite's influence a certain number of individuals fail to develop the setae altogether or develop them only to an insignificant extent, but others during the appropriate season may develop them quite fully. I find no definite correlation in the degree of feathering and condition of the gonad, and evidently there is a good deal of variability in this respect.

2. Internal Organization.

I find no appreciable reduction of the liver in parasitized individuals, such as appears to characterize some other cases of parasitization by *Epicarids*, and the only effects to be considered under the present heading are therefore those on the gonads themselves or their ducts.

(a) The Adult Male Reproductive Organs.

(i) Normal Structure.

The testis of *Upogebia littoralis* consists of a pair of sacculated tubes. They begin anteriorly in the dorsal region near the hind end of the stomach and in front of the heart, and are connected not far from their front ends by a transverse bridge across the middle line. They then pass down into the lateral region of the body at the sides of the liver and connect with the vas deferens. This is a simple unconvoluted tube with a moderately thick wall of longitudinal muscle-fibres, in which, as seen in transverse section, the nuclei form a zone towards the periphery, and an outer sheath of circular muscles. The portion which connects with the testis is considerably narrower than the rest, but the actual wall is considerably thicker, though of similar structure. There is no definite specialization of a terminal ejaculatory part. In the breeding season the vas deferens is full of spermatozoa. The masses of spermatozoa embedded in the glandular secretions of the duct emerge from the latter in the form of a solid cord.

The general histological picture presented by sections is rather closely similar to that of sections of the testis of the allied hermaphrodite form *Calocaris*, as figured and described in detail by Runnström (1925), though the macroscopic structure of the gonad is different and the several stages of gametogenesis also present some differences in detail. In *Calocaris* the testicular portion of the gonad consists of a tube with a lumen on the dorsal side and having projecting from its wall many follicles, in which the germ-cells develop. In *Upogebia* there are no definite follicles, but the tube, as already described, is merely somewhat sacculated. In section the germ-cells are seen

to form a mass on one side of the tube, leaving a clear lumen on the other. Inside the thin 'tunica propria' the gonad is lined by a syncytial layer with irregular, more or less oval, granular nuclei, the follicle nuclei (Follikelkern) of Runnström, while the main mass of the cellular contents consists of the germ-cells, amongst which prolongations of the syncytial layer with occasional nuclei can be detected here and there, evidently performing a supporting and nutritive function. The process of spermatogenesis is quite straightforward and its general course can be easily followed. The main stages are indicated in the figures of Plate 1, and for present purposes a more detailed description is unnecessary.

In the young testis of *Calocaris* follicle formation begins posteriorly and gradually extends forwards, but in the older testes several zones can be distinguished, in each of which the follicles have developed in the way described, so that in each zone the posterior follicles contain the most advanced stages and the anterior the youngest. During the period of activity of the testis the discharge of sperm from a ripe follicle is followed by its regeneration, which is accomplished by some of the residual spermatogonia (which have remained in the central tube) pushing into its base, multiplying, and thus giving rise to a new follicle bulging out at the side of the old one, which gradually collapses. In a mature testis the follicles of the hinder zones have been regenerated two or three times. In *Upogebia* something of the same kind occurs. In an intact mature testis two or more zones can ordinarily be distinguished in either half, in each of which zones the youngest stages are in front and the oldest behind. By the time one generation of cells is in the spermatid stage clumps of residual spermatogonia left over from the last proliferation have begun to multiply, and these in due course give rise to another generation of spermatozoa. When the ripe spermatozoa are formed they are simply freed into the lumen, which is directly continuous with that of the vas. Throughout the period for which I have material, namely, from early March to the end of September inclusive, spermatogenesis is going on actively, and in almost any gonad all stages can be found from spermatogonia to mature spermatozoa.

(ii) The Development of Oocytes in the Testis.

A noteworthy feature is that amongst the male germ-cells there not uncommonly occur isolated larger cells having all the appearance of oocytes. The nucleus shows a very definite nucleolus and the cytoplasm usually stains rather more darkly with Ehrlich and Eosin than that of the spermatogonia or other stages in male gametogenesis. These cells seem to be most frequently observable amongst spermatocytes or spermatids, but they may also be found amongst spermatogonia. Commonly they occur singly, but it is not very unusual to find them in couples and I have once observed a group of three.

It is evident that when the accompanying normal male cells reach maturity and are freed as spermatozoa these cells are left free in the lumen of the tube, in which situation they may sometimes be found and where they degenerate. They are more frequent in some gonads than in others, though never very numerous. The largest number I have observed is about twenty-four in one half of the gonad, and usually there are considerably fewer, but they are rarely quite absent. They occur in testes fixed in all the months of the period of study, and I find no convincing evidence of any definite seasonal variability in frequency. In my series it happens that the gonads in which they are most frequent belong to the earlier part of the period, but I do not think this is significant. They do not all attain the same size. Cells which have accompanied the contemporary normal cells through their full developmental cycle and become detached with them into the lumen of the tube may still be quite small. The largest I have seen is one about $55\ \mu$ across, amongst spermatocytes.

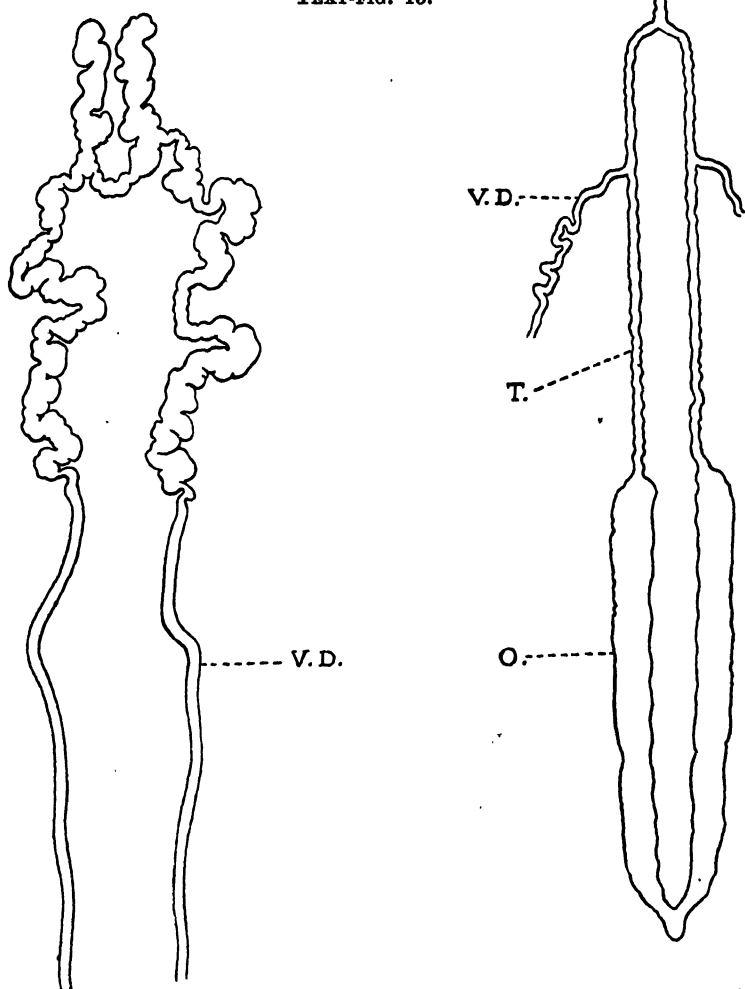
Though these cells are not infrequent amongst the more advanced spermatogonia, I have not succeeded in identifying them with certainty amongst the youngest or primary spermatogonia. I am thus not able to determine positively whether they arise by modification of spermatogonia or whether, as on general grounds is perhaps more likely, they are really distinct from these from their origin. Potts (1906) believed that he could trace the egg-cells which arise in the testes of *Eupagurus*

parasitized by *Peltogaster* from spermatocytes, but this seems to require confirmation. Runnström has shown that in the hermaphrodite gonad of *Calocaris* oogonia and spermatogonia are visibly distinct from the outset, the oogonium having rather less cytoplasm than the spermatogonium, and a rather larger nucleus, and also differing from it somewhat in the distribution of the chromatin in the nucleus. He has also demonstrated that the limited number of egg-cells which occur actually in the testicular region arise, not sporadically at any point, but from a perfectly definite and localized band which produces nothing else. In *Upogebia* the visible differences between young male and female cells appear to be less marked, and a comparison of ovaries and testes does not bring to light any sufficient difference in the nucleus-cytoplasm ratio between young oogonia and spermatogonia in this form to give grounds for believing that they could be differentiated by this criterion where they occur together. It certainly is possible to find amongst young spermatogonia occasional cells with nuclei rather larger than the normal, though not otherwise noticeably different from the rest, and others may be seen occasionally which suggest a transition from these to the definitely oocyte-like cells now under consideration. The connexion, however, can hardly be considered definitely established.

(iii) A Comparison with other species of *Upogebia*.

The condition now described in *Upogebia littoralis* naturally invites comparison with that found in the Japanese *Upogebia major*, described many years ago by Ishikawa (1891) and often quoted since. The testis of *Upogebia major* differs considerably from that of *Upogebia littoralis* in that it has on each side a posterior extension into the abdomen comparable to the abdominal extension of the ovary. In all of twenty specimens examined by Ishikawa these abdominal extensions of the testis contained well-developed oocytes. He mentions that sometimes well-developed egg-cells are also found in the first part of the testicular sac, and in his

TEXT-FIG. 15.



1.

2.

Male reproductive organs of *Upogebia littoralis* and *Upogebia major*. 1. *Upogebia littoralis*; 2. *Upogebia major* (after Ishikawa). V.D., vas deferens; T., testicular region; O., ovarian region.

figure he shows such an isolated egg-cell amongst male germ-cells. We have here a condition directly comparable with that which I now describe in the European species, but in *Upogebia littoralis* there is nothing comparable to the posterior extension of the testis of *Upogebia major*; the testis ends where the vas deferens comes off.

In order to ascertain the conditions in another species I obtained from the Plymouth Laboratory several specimens of the British *Upogebia stellata*. These were received on March 3, having been collected at Salcombe a day or two previously. I found that in the males of this species a well-developed abdominal extension of the testis exists, but it is not exactly the same as that of the Japanese form. For the greater part of its length, at any rate at the season in question, it is extremely slender, and it is only at the extreme posterior end that it is expanded for a few millimetres into a wider structure. This terminal expansion, in each of the two males examined, contains large and well-developed oocytes. In the rest of the gonad, as would be expected at the time of year, spermatogenesis is almost completely in abeyance. The whole tube is much attenuated and the cells have a loose and rather jumbled arrangement, contrasting sharply with the compact and orderly arrangement of the cells when spermatogenesis is going on actively, and in fact closely recalling the appearance presented by gonads of *Upogebia littoralis* undergoing atrophy under the influence of Gyge. This is evidently the appearance assumed during phases of partial or complete cessation of spermatogenetic activity. There are a few spermatozoa in the lumen of the tube in the abdominal as well as the thoracic part, and a fair number in the vas deferens, evidently persisting from the previous season. In the two testes which I have examined I have not observed isolated cases of egg-cells amongst sperm-cells, such as occur in the other two species, but from analogy with these latter it is probable that they sometimes occur. The oocytes in the terminal expansion of *Upogebia stellata* are larger than those usually found in *Upogebia littoralis*. Those of *Upogebia major* may even form yolk, but I found no evidence of yolk formation in

my specimens of *Upogebia stellata*, nor does this occur in the isolated oocytes in *Upogebia littoralis*.

Another point of interest in connexion with *Upogebia stellata* is that in one of the males examined there was present at least on the right side a slender strand running from the testis to the region of the base of the third walking leg, i.e. in the same position as the oviduct in a female. No trace of a corresponding orifice could be detected externally, but I was able to demonstrate by sectioning that the internal structure just referred to, slender as it was, nevertheless contained a definite lumen. It can thus be safely identified as a rudimentary and non-functional oviduct. Some time after ascertaining these facts I found that substantially similar conditions to those above described had been discovered by Runnström in three males of *Upogebia stellata* which he examined in connexion with his study of *Calocaris* and which were obtained from the same source as mine. The conditions were not identical in his three examples. In No. 1 there were membranous areas suggestive of closed female pores on the coxopodites of pereiopods III, and similar but smaller ones on pereiopods IV. The internal structure is not described. No. 2 had definitely pierced openings on pereiopods III, agreeing exactly with oviducal pores. Dissection disclosed on the right side two thin strands, each shown to contain a definite lumen, running down to pereiopods III and IV respectively. The anterior one evidently connected with the corresponding external pore; the other must have ended blindly. No. 3 was merely noted to have got supernumerary genital orifices without precise details, and the body was not preserved. The internal organs showed a strand comparable to those above described running down to pereiopod III on the left side.

It is thus seen that in *Upogebia stellata* the tendency to hermaphroditism is not confined to the production of oocytes in the posterior part of the testis, but is displayed also in the frequent development of supernumerary genital ducts and apertures, though the extent to which these accessory structures are differentiated is subject to considerable individual variability. The fact that in addition to ducts and apertures agreeing

with those of the female, similar structures may be at least partially developed in connexion with the fourth pair of thoracic limbs is deserving of note, and might be held to suggest that we are dealing here with a tendency to segmental repetition of a normally unisegmental organ rather than to the development of a specifically female structure in the male. However, judging from the few specimens examined, it appears that the most usual condition is for the structures in question to be present only in the oviducal segment, and that when they are present also in the segment behind they are not so well developed. The conclusion that a definite tendency to 'femaleness' is here involved thus appears to be justified and is in accordance with what has been described with regard to the germ-cells.

Runnström's account of the short ovarian region at the hind end of the testis is in agreement with mine, and it is only necessary to add that in one of his specimens the oocytes actually contained yolk, which was not the case with those which I examined. The time of year at which his material was obtained is not stated.

In two males of *Upogebia deltura* structures apparently representing closed homologues of the female genital pores were also observed by Runnström, and on at least one side of one of the individuals there was a connexion with the gonad in the form of a slender strand such as has been described in *stellata*. He also describes his observations on several males from Naples, which were in fact *Upogebia littoralis*, but here a little confusion is introduced by his failure to recognize that *Upogebia stellata* and *Upogebia littoralis* are perfectly good and distinct species separated by definite morphological characters.¹

Regarding them as all one species he is at some pains to explain the fact that neither supernumerary genital pores nor a posterior ovarian region of the testis could be detected in the

¹ See, for example, De Man, 'Capita Zoologica', 1927. It may be added that any one familiar with the two forms in life would certainly never suppose them to be the same species, *Upogebia stellata* being whitish, with fine stellate orange spots, which give it a more or less orange tint as a whole, while *Upogebia littoralis* is predominantly a dull green.

Neapolitan examples. He states first that no portion of the testis could be observed in the abdomen of either of the males examined, but goes on to say that in one of them he 'was able to observe in the toto-preparation how the testis became narrowed and one piece merged in the abdomen with a sterile portion, in which all trace of germ-cells was lacking'. In view of the account which I have already given of the testis of *Upogebia littoralis* it is almost unnecessary to add that if this description of Runnström's is really correct, and he has not been misled owing to faulty preservation of the specimen, the condition described is entirely abnormal, no such abdominal extension, sterile or otherwise, occurring in the testes of normal males of this species.

Runnström makes the suggestion that the ovarian region atrophies when the testis reaches its maximum activity, and that the Neapolitan males examined by him had passed this stage. But the absence of supernumerary ducts and pores still remains a point of distinction from the British animals, and he seeks to account for the observed differences by supposing that there is a geographical variation in the sexual organization within the species, comparable to that described by Cuénot in *Asterina gibbosa*. Since, however, *Upogebia stellata* and *Upogebia littoralis* are without doubt distinct species, these suggestions are off the mark and need not be considered further.

The question whether there is any seasonal variability in the extent to which egg-cells are developed in the testes of *Upogebia* is of some importance. In the Sandhopper *Orchestia* Smith (1906) has shown that the egg-cells are only normally present in the non-breeding season,¹ so that their development

¹ Smith's conclusions were subsequently challenged by C. L. Boulenger (1908), who worked on the same form, *Orchestia deshayesii*, at Naples, but obtained different results. In the considerable material which he examined he found ova normally present only in the testes of immature males, which led him to suggest that Smith did not sufficiently distinguish between old and young males in his observations. Smith, however, is quite explicit on this point. Speaking of 'very numerous' specimens of *Orchestia deshayesii* collected during the months December to March, he says (1906, p. 92) that he found on dissection that

would appear to be connected with a particular type of metabolism associated with that period. Apparently when spermatogenesis begins actively the ova degenerate. Ishikawa does not state at what time of year his specimens of *Upogebia major* were obtained, but his communication is dated 'Tokyo, May 1890'. He concludes, no doubt correctly, that the egg-cells must degenerate at a 'certain season of the year'. In *Upogebia littoralis* I have shown that a limited number of egg-cells may be found in the testes right through the breeding season, so that the conditions obtaining while active spermatogenesis is in progress are not absolutely incompatible with the existence of these female cells.

It is possible, of course, that during the period of active spermatogenesis few or no new egg-cells arise, but I have seen one amongst spermatogonia in a testis fixed at least as late as the third week in May. At present I have no mid-winter material to show whether or not the egg-cells are more numerous at that season, a point of considerable interest which I hope to settle before long. At any rate, when active spermatogenesis is resumed the egg-cells do not thereupon disintegrate. On the contrary, as I have shown, they persist until they are detached into the lumen with the associated cells and degenerate there. In *Upogebia stellata* they seem to degenerate in situ, perhaps at the onset of the breeding season. In one of my two series of sections, in addition to oocytes of normal appearance, there are others in various stages of degeneration. In an advanced stage the section shows an irregular mass representing the degenerating cytoplasm of several oocytes in which cell boundaries are no longer distinguishable and in which are several clearer vesicles representing the nuclei, each containing a sort of halo of granules derived from the chromatin.

'more than half the males whether fully developed or not and of all sizes (spaced words mine), exhibited the ova in their testes'. This is quite clear and definite and can hardly be explained away in the manner suggested by Boulenger. A more extended study of the whole question is desirable. It appears probable to the present writer that some essential facts are probably lacking, which when discovered will reconcile the seemingly contradictory experiences of these two observers.

(iv) The Effects of Parasitization.

The condition of the testis in parasitized males shows a wide range of variability, from one in which it is only very slightly reduced and normal spermatogenesis is still going on vigorously to one in which no trace of the organ can be detected. The proportion of specimens in the latter category decreases with increasing expertness in dissection and as experience gives increasing aptitude in detecting minute vestiges, but there still remains a not inconsiderable number in which it is impossible by the most minutely careful dissection under a dissecting microscope to detect any trace of the gonad at all. Even the fully developed organ has a semi-translucent appearance which makes it none too easy to follow, and when it is much reduced the difficulty is of course greatly increased. I have found the difficulty is minimized by dipping the whole body (after removal of the carapace) for about a minute in Bouin, the fixative chiefly used for these gonads, which gives a certain opacity to the organs and greatly facilitates dissection. The testis can then be dissected out comparatively quickly and the fixation in Bouin completed.

In those gonads which are reduced but recognizable there is no uniformity in the mode of reduction. The testis may be relatively more reduced than the ducts or vice versa and the degree of reduction is not invariably absolutely identical on opposite sides of the same animal. In several specimens which I have dissected the anterior end of the testis is still fairly developed, while the posterior end has undergone marked reduction.

As reduction proceeds the testicular tube becomes more and more attenuated and loses its sacculations, but the macroscopic structure of the organ is only a rough and somewhat uncertain guide to its histological condition. A testis which is well developed and not much below normal size will generally show a certain amount of normal spermatogenesis with quite numerous spermatozoa in the lumen and ducts, but even this is not invariable; spermatogenesis may be found to have been checked in a gonad which externally appears quite well

developed. Testes which have undergone considerable reduction, but are still recognizable in their entirety, may have completely ceased to produce spermatozoa or may still show normal spermatogenesis in parts, while those which are reduced to vestigial proportions, though ordinarily completely degenerate, may occasionally show some normal spermatogenesis going on right up to the formation of apparently normal spermatozoa. Degeneration sets in locally and not uniformly throughout the organ; indeed normal spermatogenesis and an advanced condition of degeneration may coexist in adjacent portions of the tube. The impression conveyed by the examination of a large number of sections is that degeneration at any given point involves first the older cells, the younger stages being less sensitive. In the later phases recognizable spermatogonia persist after all the other cells have aborted completely and they are the last to go.

Although continued sperm-production is quite common in parasitized animals I have never examined a case where some local reduction and degeneration was not observed, though it may be inconsiderable. A very general feature of parasitized testes is that the cells tend to acquire a looser arrangement, no longer presenting that orderly and compact formation which is so characteristic of the normal organ. In one or two instances I have come across parasitized males with the external effects of parasitization fully shown, in which a considerable portion of the testis nevertheless displayed a histological structure indistinguishable from the normal, the germ-cells showing just the same compact and regular arrangement as in the testis of an uninfected animal; but this is unusual. As a rule, even when spermatogenesis is proceeding quite actively, a section of a parasitized testis can be recognized with considerable confidence.

As degeneration proceeds, the normal spermatogenetic stages disappear, though a few spermatogonia commonly persist for a long time, the cells become more and more irregular and scattered in their arrangement, and aborted cells containing rounded globules of a deeply staining substance, evidently a degeneration product of chromatin, occur freely. Finally

sections show little but degenerating cells, cell-debris, and pigment granules.

It is not uncommon for sections of parasitized testes to show the germ-cells extending completely round the tubule, enclosing a central lumen, instead of being confined to one side with the lumen on the other. Occasionally the lumen is completely occluded for a short distance. Instances may also be observed occasionally where there appears to have been merely a more or less marked local thickening of the (apparently syncytial) lining of the tube (Follikelplasmodium of Runnström) without any fresh germ-cells being produced. Such cases of local hypertrophy are to be regarded merely as a part of the general disorganization of the reproductive functions resulting from parasitization.

In at least two cases which I have examined the vas deferens has developed a cuticularized lining. This recalls the condition described by Potts (1906) in *Eupagurus* parasitized by *Peltogaster*, in which the later stages of parasitization are characterized by an excessive production of chitin in the male ducts. In *Eupagurus*, however, there are special spermatophores with a chitinous cap, and the irregular masses of chitin appearing in the ducts are stated to be derived from the cells producing these caps. In *Upogebia* there are no such definite spermatophores, and the chitinization of the duct lining in parasitized specimens is only an occasional occurrence. It does not seem possible to connect it with any particular phase of parasitization and it is not confined to the late stages, since it has occurred quite extensively in one case where spermatogenesis is still proceeding normally, with only a very little local degeneration, and the duct is full of spermatozoa.

The development of egg-cells in the testes of infected individuals is quite irregular. The proportion of individuals in which they occur is hardly greater than in normal animals; but when they are present they are commonly, though not invariably, much more numerous, and their growth and development proceeds farther than in normal testes. That is to say, the tendency to produce these cells, which exists in a small degree in many or most normal *Upogebia*, is markedly

accentuated in a not inconsiderable proportion of parasitized specimens. While in a normal testis these cells appear in ones and twos scattered at comparatively rare intervals amongst the male cells, in that of a parasitized example they commonly arise in some numbers together. Though their ultimate fate must in all cases be degeneration and disintegration, they grow to a larger size than in uninfected animals and may even produce a small quantity of yolk granules, which I have never found in normal specimens. Occasionally a whole tract of tubule may be converted largely, if not entirely, into ovary. It is difficult to state positively that the younger cells in such modified regions are definitely oogonia rather than spermatogonia—but on the whole I think that in the more advanced cases the conclusion is justified that definitely male elements have really been locally completely suppressed. The nuclei of the younger cells in these cases appear ordinarily rather larger than typical male cells in the same stage, with the chromatin granules more clearly defined and the rest of the nucleus rather clearer.

No grounds are afforded for regarding these modifications as a constant or necessary accompaniment of any particular phase of parasitization. So far as can be judged the testis of a parasitized male may undergo complete degeneration without at any time developing egg-cells. On the other hand, a region apparently completely modified into ovary may occur in a testis in other regions of which spermatogenesis is still going on and spermatozoa being formed.

In one or two parasitized males which I have dissected the reduced testis has shown slight abnormalities of form. Thus, in one case the two halves meet in a V anteriorly instead of being united by a transverse bridge some millimetres from their front ends. In two cases I have found a short posterior extension or diverticulum of the testis extending a few millimetres beyond the point of connexion of the vas deferens with the organ. Such an extension was present on one side of the slightly abnormally shaped testis just mentioned and in the other case one was present on each side. The occasional development of these posterior extensions in parasitized examples is curious, for although they do not reach into the abdomen they at once recall

the longer normal posterior extensions of the testis of *Upogebia major* (cf. p. 48) or of the ovary of the female in the present form, and I was not unprepared to find that they were ovarian in their histological structure. This, however, is not the case. Both the testes in which they occur are in a rather advanced stage of degeneration, only a few spermatogonia persisting. The extensions in the last described specimen have the same structure as the rest of the organ, with no oocytes or any ovary-like development. In the other the single extension also shows a structure much like that of the rest of the organ. As in other parts of this testis, some of the cells, which seem to be degenerate spermatogonia, are rather suggestive of oogonia or small oocytes, but the appearance is inconclusive, and at any rate the structure is not definitely ovarian.

(v) Exceptional Conditions.

When a large number of specimens are examined, conditions are occasionally met with differing from those dealt with above. Two such are described here :

1. Unparasitized male with female appendages.—This might have been either an individual abnormality or a formerly parasitized specimen in which the Gyge had been lost naturally, but there was no swelling of the branchiostegite to indicate the former presence of an *Epicarid*.

The microscopic appearance of the gonad agrees closely with that of some parasitized testes. Spermatogenesis is progressing normally to the formation of spermatozoa, but numerous oocytes are also present, many more than in any unparasitized gonad which I have examined. On the whole I am inclined to regard this as a case of natural recovery from parasitization (cf. p. 69).

2. Parasitized males without female appendages.—I have met with two or three cases of parasitized males without any trace of female appendages and have examined the gonads of two. At first sight it might be supposed that these were exceptional cases of late parasitization, in which the appendages had not yet appeared. Such a supposition,

however, is not borne out by a microscopic examination of the testis of the first specimen, though the second might perhaps be so interpreted.

In the former the gonad is greatly reduced; only a small vestige could be detected and the structure is quite degenerate. I regard this as an individual abnormality, an isolated case in which, owing to some presumably constitutional difference from the normal the animal has failed to respond to the presence of the parasite in the normal way, i.e. by developing female appendages.

The second case, a specimen of 14 mm. carapace length, is quite different. The gonad was scarcely less well developed than in a normal animal and normal spermatogenesis was in progress. These facts are consistent with the assumption that this is a case of recent infection of a fully adult individual. On the other hand, the chelae are markedly reduced (length, 10.5 mm., breadth, 3 mm.), which would be surprising if the parasite had only lately established itself. The latter was also not noticeably smaller than the branchial chamber could accommodate. It must be understood that the absence of any visible effect on the gonad cannot in itself be taken as absolute proof of recent infection, for I have examined at least one parasitized specimen in which sections of the greater part of the testis were almost or quite indistinguishable from the normal, which was nevertheless fully modified externally. Finally, though its bearing, if any, on the associated peculiarities is doubtful, it should be mentioned that the structure of the testis was abnormal on one side. The testicular tube at or near the origin of the vas deferens had an outgrowth or duplication which appeared to connect again with the vas farther back. The precise relations of this outgrowth and the vas could not be made out satisfactorily in the dissection and cannot be properly reconstructed from the sections owing to some displacement of the parts from their original positions, but at any rate a structural abnormality existed.

It is not possible to arrive at a definite conclusion as to the nature of this isolated case. It may be said that it is suggestive, up to a point, of parasitization exceptionally late in life, but it will be seen that there are some features which do not allow

this conclusion to be drawn with any certainty and in fact are distinctly unfavourable to it.

(b) The Adult Female Reproductive Organs.

(i) Normal Structure.

The general form of the ovary is similar to that of the testis, but it is prolonged backwards through the abdomen almost to the extremity of the body. The ova give it a characteristic green colour. The ripe organ occupies a large part of the body-cavity and the oviducts may be found distended with eggs almost down to the external apertures. The ducts are short and very thin walled and when not full of eggs are difficult to see in a dissection. In a female which has recently laid, the ovary is of course much reduced and contains only small and quite immature oocytes and earlier stages, while the oviducts are seen in sections as very attenuated and thin-walled tubes. The oviducal apertures are more conspicuous than the genital pores of the male. There is evidently some variation in the time of attainment of sexual maturity, but a few individuals of as little as 8-9 mm. carapace length may be found bearing eggs.

(ii) The Effects of Parasitization.

In general the effect of the parasite on the female gonad is more drastic than in the case of the male. This is natural, since the production of the richly yolked eggs involves a greater drain on the animal's resources than the production of spermatozoa. In the large majority of parasitized females no trace of the gonad can be recognized on dissection, but a very few occur in which a reduced, but complete, ovary can be seen and dissected out. The organ in such cases is found to be an ovary in a state of reduced activity, but not otherwise abnormal. Well-yolked eggs are present, but probably none reaching a fully mature condition, while some are obviously degenerating. While there is no obvious reason why a certain number of the less affected parasitized males should not breed, it may be asserted with considerable confidence that parasitized females are never capable of doing so.

(c) The Development of the Reproductive Organs.

It has been shown that *Gyge* differs from such other crustacean parasites as *Sacculina* in that the host is almost exclusively parasitized when extremely young, so that instead of the effect of the parasite being brought to bear on a fully mature gonad or at least upon one well advanced in development, the organ pursues its development from an early stage under the parasite's influence. This being so it appeared desirable to study the early stages of development of the reproductive organs and to compare them with those of parasitized individuals. Such a study was carried out by means of serial sections of the youngest animals obtained. In these the chitin is so delicate that, although a certain number of partial failures occur (even when the membrane between carapace and abdomen is pierced to facilitate entry of the fixative), fixation is generally quite good enough for all ordinary purposes, though doubtless not for detailed cytological work.

It must be admitted that the somewhat lengthy process of preparing, sectioning, and examining a series of stages of young *Upogebia* has been in some ways a little disappointing, chiefly owing to the variability not merely of the parasitized animals (which was to be expected), but of the normals with which they are to be compared, which renders the making of any very precise comparison difficult. The results, however, help to complete the picture already given.

According to Butschinsky (1894) the gonad can be distinguished in the latest stages of embryonic development as a paired rudiment close to the mid-gut under the heart. Here certain mesoderm cells increase rapidly in circumference and give rise to the rudiment of the genital organ. Miss Webb (1919) finds indications in *Upogebia stellata* and *deltura* that an external sex differentiation starts very early, perhaps from the beginning of larval development (cf. p. 17).

(i) Normal Male.

In the youngest male I have examined, of 8 mm. carapace length, the testis has already assumed its definitive form,

though it is still minute. A very small vas deferens can be traced, passing downwards just laterally to the liver. It appears to be hardly more than a rudiment; a thin strand of cells, which does not appear yet to have acquired a continuous lumen. There are indications of a lumen in the region where it is passing down through the ventral muscle mass. It can be followed on through the base of the last pair of legs to its connexion with the exterior. The genital pore is indicated externally, but it is doubtful whether a definite aperture is pierced at this stage.

By 4 mm. carapace length a striking increase has taken place in the size of the gonad, which is now a conspicuous structure in the sections. Spermatogonia are now recognizable. The vas deferens is quite well developed, though commonly still slender and rather thin walled. The ventral section is rather wider and thicker walled than more dorsally.

One of the most interesting facts which have emerged from the study of these young animals is the extraordinarily early period at which sexual maturity is attained by certain individuals. The usual condition of the gonad in males of 4 mm. carapace length has just been described; there are no stages of spermatogenesis beyond young spermatogonia. But in two individuals of this length which I have sectioned, spermatogenesis is proceeding through all its stages up to the production of spermatozoa, quantities of which are present in the lumen of the testis and in the duct. Sperm-production at such a very early age is remarkable, and so far as I am aware had not been described in any other Decapod, though Orton (1920) records a comparable case in the barnacle *Conchoderma virgatum*, in which a small proportion of individuals aged not more than five weeks were found to be breeding, and refers to parallel instances in Mollusca (e.g. *Patella* and *Meleagrina*), Echinoderms (*Echinus miliaris*), and various other invertebrates.¹

A badly fixed specimen of approximately 5.5 mm. carapace length is also producing spermatozoa, and from about 6 mm. onwards all those examined are sexually mature, though it is improbable that they actually breed until somewhat later.

¹ I am indebted to Dr. J. R. Baker for directing my attention to this paper.

(ii) Parasitized Male.

The series of parasitized males which I have examined by the method of sectioning includes stages from 3.5 mm. to 9 mm. carapace length, that is to say, it ranges up to a stage somewhat beyond that at which the dissecting out of the gonad and ducts becomes a reasonable proposition. The parasitized material which I have been able to study therefore ranges from 3.5 mm. carapace length up to the maximum size.

In the young parasitized animals the variability in the development of the gonad noted in normal material is not unnaturally somewhat exaggerated, but in general it is clear that the whole process of development and differentiation is markedly retarded, the gonad itself appearing to be more sensitive during the period of development than the ducts, since it is usual in these young animals to find the latter at any given stage more advanced and better developed than the former, which is not noticeably the case in normal males. A description of some typical stages will give an idea of the state of affairs found.

In a specimen of 3.5 mm. carapace length no rudiment of either gonad or duct can be traced.

In one of approximately 4.5 mm. there is still no recognizable trace of a gonad, but a rudiment of the vas deferens can be traced from the region of the genital pore in the limb up to the sides of the liver-mass, where it is lost. In the body it shows definite indications of a lumen, at least in parts. In the limb it is simply a thin, solid strand of cells. In another of the same size the ducts are distinct in the limbs, but appear to be solid and not to connect with the exterior. They can be traced up the sides, and a minute strand of cells representing the rudiment of the gonad can be traced with certainty in places and more doubtfully at intermediate points.

In a specimen of 6 mm. the duct is quite fairly developed, with lumen and opening to the exterior, and the gonad rudiment, though small, is easily traceable throughout.

In another of 7 mm. the duct has either undergone a regression or has never developed so far as in the preceding, and the gonad

consists of almost the most attenuated vestige that could be recognized as such. In a preparation of another animal of the same size, including only the anterior part of the body, the gonad is seen to be quite conspicuous and of fair size, though quite degenerate in structure.

In sections of 9 mm. specimens I have found minute testes, recalling in appearance the early rudiment of the organ in the youngest normal males examined, and others rather more developed, showing in at least one case darkly staining cells shrunk apart from one another, similar to cells which I interpret in normal material as spermatogonia or other stages imperfectly fixed. Local irregularities in development now become more obvious, and a testis which is of fair size in one or more regions may become indistinguishable in others. The condition of the duct is also variable; it may be quite well developed or much reduced and in some cases apparently disconnected from the exterior. Such very attenuated ducts would probably not be recognizable in a dissection.

I have not met with a case of definite spermatogenesis in any parasitized male of less than 8 mm. carapace length, but I have found it in progress in an example of this size, in which spermatozoa are present in the lumen of the organ.

(iii) Normal Female.

My normal female material does not include individuals of quite such small size as in the case of males, but one of 4 mm. carapace length has the ovaries still extremely small and rudimentary though the oviducts are quite well developed. There is evidently, as in males, some variation in the rate of development. Small oocytes appear between 4 and 6 mm. carapace length. A specimen of 8 mm. has oocytes larger than a 6 mm. example, but still immature, though some examples of this size can be found bearing eggs. 9 mm. specimens with eggs are not rare and a non-ovigerous example which I have sectioned is almost or quite ready to lay. If the conclusions tentatively indicated on p. 12 are sound, such animals would be breeding at the age of approximately one year. It is probable that some,

possibly the majority, do not breed until they are two years old, when probably they average about 12 mm. carapace length.

(iv) Parasitized Female.

Parasitized females differ from parasitized males in that whereas in a large number of the latter the testis is developed to some extent, in the former the development of the gonad is normally completely checked. In young parasitized females the ovary is either not traceable at all in sections or appears to be represented by a minute and scarcely recognizable vestige. The oviducts, on the other hand, can generally be traced for some distance inwards from the genital pore, sometimes as a mere strand of cells, sometimes as relatively quite well-developed ducts passing inwards and generally 'petering out' and becoming lost in the region of the liver. Even when a presumed vestige of the gonad can be traced it is not always possible to establish any connexion between it and the reduced inner ends of the ducts. The relative persistence of the latter may be regarded as correlated with the fact that the genital aperture in the chitin of the coxopodite remains always distinct, even in the most highly modified females. Judging from the young material now described it is probable that vestiges of the ducts persist also in the older animals or many of them, but they would hardly be recognized in a dissection.

3. Some Supplementary Considerations.

(a) Correlation of Modifications.

It will be clear from the foregoing account, and more particularly from that of the changes undergone by the testis in the male, that there is not only considerable variability in the extent of the modifications undergone by the reproductive organs, but also a not inconsiderable qualitative variation—e. g. in the production or non-production of oocytes, the occasional cuticularization of the lining of the duct, &c. It will be further evident that the several changes observed, although they must be regarded as all expressions of the same set of physiological causes at work, must proceed to a very large extent independently of one

another. The gonad may be reduced more than the duct or vice versa, or one part of the gonad more than another. It has been shown that the macroscopic appearance of the gonad and the degree of its reduction in size are not safe guides to its internal condition, and, further, that the internal changes themselves are not at all closely correlated, but may occur in varying combinations.

A more precise study of a series of parasitized males undertaken with the express object of inquiring into this particular question of correlation of modifications has not only confirmed the above conclusions with regard to the internal changes, but has also failed to demonstrate any definite correlation between any of these and the external modifications.

Amongst animals of the same size one with the testes in a condition of extreme degeneration may have chelae less reduced than another in which the gonad is still quite well developed and still producing spermatozoa. Again, the 'feathering' of the first pair of abdominal appendages in parasitized males does not appear to be associated with any particular condition of the testis. It is true that these appendages are fully feathered in both of the two cases where egg-cells are most extensively developed, but the condition is not peculiar to examples containing such cells in the gonad. It occurs also in association on the one hand with extreme degeneration of the testis, and on the other with quite a considerable amount of sperm-production. Indeed, in at least one case the appendages are moderately well feathered in a male in whose testis spermatogenesis is proceeding normally almost throughout, with only vague indications of the beginnings of a little local degeneration and no oocytes.

Since all the modifications must be ultimately dependent on the same causes it would be surprising if there were no correlation whatever between them; but such correlation as may be supposed to exist is manifestly of an extremely loose and imperfect character and of an order which only a very minute consideration of a very large series could detect. A similar lack of correlation between the several modifications has been observed by Potts (1906) in the case of *Eupagurus* and *Peltogaster*.

(b) The Question of Recovery from Parasitization.

The question of what happens to the gonad if the host is freed from the parasite and whether the external modifications show any regression to or towards the normal is one of considerable interest which cannot be passed over without mention, although I am not at present in a position to throw much direct light upon it.

It may be recalled that in the case of *Inachus* and *Sacculina* Geoffrey Smith (1906) showed that although the parasitized male crabs do not produce ova in the testis while bearing the *Sacculina*, fully modified individuals, on being freed from the parasite, regenerate a hermaphrodite gonad. In the case of *Eupagurus* and *Peltogaster*, described by Potts (1906), egg-cells occur commonly in the testes of infected males, though not in normal ones. These cells were found to be still present in the gonads of crabs which had been freed from the parasite several months before, and in at least one case appear to have grown larger than in infected testes; but apparently no animals were kept long enough for the gonad to regenerate completely, and the question whether or no the final result would be a completely hermaphrodite organ as in *Inachus* is left open.

With regard to modifications of the appendages some experiments by Potts show that these persist in *Eupagurus* for at any rate a considerable time after removal of the *Peltogaster*, but it is not proved that they are permanent. In a modified male *Eupagurus bernhardus*, from which a specimen of the Bopyrid *Phryxus* (= *Athelges*) *paguri* was removed, Giard thought he detected an incipient return to the unmodified condition of the appendages when the crab moulted a month after removal of the *Epicarid*, but this observation is rather indefinite.

During my first visit to Naples, the only one during which I was working at the Zoological Station for a sufficiently long period for the purpose, an attempt was made to ascertain the results of removing the parasite. Owing to the heat, uncertainties as to the natural food, and some other practical diffi

culties, I was unable to carry on the experiment long enough to obtain results of much value. A number of *Upogebia*, relieved of their parasites, were kept for some seven or eight weeks and a few even rather longer, and during the period of observation moulted probably more than once, though an exact record of the periodicity of moults was not kept. In some the swollen branchiostegite seemed to show distinct signs of reverting to the normal condition, but in none was an appreciable change in the chelae or abdominal appendages detected nor, in the few individuals examined, was any definite evidence found that a reduced testis was being regenerated. To obtain conclusive results, whether positive or negative, the animals ought to be kept for several months at least. My own experience in keeping them for shorter periods does not suggest that with a little trouble and ingenuity this should present any insuperable difficulties.

There is no evidence that in nature loss of the parasite is anything but an exceptional occurrence; normally its life-span would seem to be coincident with that of the host. I have only critically examined one probable case of natural recovery, namely, the male individual described under 'Exceptional Conditions, 1', p. 59. If this is really a case of such recovery it confirms the conclusion that the above imperfect observations suggest, namely, that the modifications have at any rate a considerable degree of permanency, for the female appendages are well developed and the testis contains more numerous oocytes than any unparasitized testis I have examined, though, if the animal really is a recovered individual the branchiostegite has had time to revert completely to the normal, unswollen condition.

4. Certain General Conditions.

From a consideration of the facts recorded in the foregoing sections certain general conclusions emerge which have not been specifically stated in the course of the account. For example, it is clear that in those parasitized males in which spermatozoa are found the latter have been produced during the period of

parasitization. The course of events would appear to be as follows :

Parasitization normally takes place at an early stage, when the testis is still in a very early stage of development. Further differentiation of the organ is markedly retarded and in some cases evidently completely checked. At a time not much antecedent to 8 mm. carapace length, or probably in some cases later, some of the less drastically affected animals may begin to produce spermatozoa. The gonad, however, is always more or less affected, as is shown by a certain loss of compactness in the arrangement of the cells in practically every case, and in the large majority by at least local degenerative changes in the histological character of the organ. These changes are clearly to a great extent progressive, and it is evident that in many animals after a period of decreasing sperm-production atrophic changes become more and more extensive and eventually spermatogenesis ceases entirely. In some individuals, however, a certain amount of sperm-production may go on for a long time and perhaps indefinitely, since spermatogenesis proceeding locally up to the formation of apparently normal spermatozoa may sometimes be observed in the testes of the largest animals. On the other hand, complete atrophy of the gonad is found in specimens of all ages, and, as described, all sorts of intermediate conditions occur. The complete, or almost complete, absence of gonad and ducts is probably in most, if not in all, cases due to failure to develop rather than to regression from a previously better developed condition.

It is reasonable to suppose that the observed variability is in part due to differences in the exact time of parasitization, for at the period when the parasite normally establishes itself the gonad is developing rapidly, so that a comparatively small interval of time may involve a quite considerable difference in the stage of development of the reproductive organs, and we may suppose that the later history of the gonad will be dependent at least to some extent on the stage which it had reached before the influence of the parasite was brought to bear upon it. Since specimens of as little as 3.5 mm. carapace length occur bearing parasites, while the example bearing the newly estab-

lished *Cryptoniscus* larva (p. 11) has a carapace length of 6.5 mm., and others about this size occur in which fixation is so recent that female abdominal appendages have not yet appeared, it can be stated that there is direct evidence that parasitization can take place at any time between these extremes. It is possible that those with the reproductive organs most completely obliterated are the earliest attacked. But I am convinced that the differences must also be dependent in large measure on constitutional differences in the animals themselves. Apart from any other consideration, the lack of correlation between the observed modifications alone implies that such inherent constitutional differences must exist.

V. REVIEW OF PREVIOUS WORK ON PARASITIC CASTRATION.

As a preliminary to any general discussion of the subject it will be necessary to consider the main results and conclusions of previous workers. Parasitic Castration has been described in all sorts of animals, but the large majority of recorded cases involve merely an imperfect development or regression of the sexual organs and secondary sexual characters. It appears to be only in the Arthropoda, or, more precisely, in the Crustacea and Insecta, that a definite change of one sex towards the other is observable, and attention will be confined to these. It will be convenient to deal with the Crustacea first, as affording the classical examples of the phenomena under discussion.

A. Crustacea.

The discovery of Parasitic Castration was made by Giard (1886), who was responsible for the introduction of the term,¹

¹ Objection has frequently been made to the term 'Parasitic Castration' on the ground that the changes are not dependent upon and do not necessarily involve destruction of the gonad, and that a false analogy is suggested with the results of castration in the higher vertebrates. The term, however, has passed into wide use, its special significance being perfectly well understood by every zoologist, and it seems to the writer unnecessary and even a little pedantic to alter so well established a designation for a set of phenomena which certainly require some name. On the contrary there is

in *Stenorhynchus phalangium*, one of the small spider-crabs, parasitized by *Sacculina*. In subsequent papers (1887 *a* and *b*, 1888 *b*) he drew attention to the occurrence of similar phenomena in sacculinized individuals of other species of *Brachyura* and in *Eupagurus* parasitized by *Peltogaster*. Comparable effects were shown to be produced in varying degrees by at least one species of the Entoniscid Isopods and several of the Bopyridae (1887 *a*, *b*, and *c*, 1888 *a* and *b*). Parasitic Castration of *Upogebia* by *Gyge* was described (1887 *b*) from a single male from Naples with the first pair of abdominal appendages developed as described in the present account.

Giard described the typical external feminization of male crabs, but considered that there was no essential difference in the reactions of male and female under parasitization, i. e. females could be modified towards the male type in respect of some characters in the same way as males can be modified towards the female type in respect of others. The changes were interpreted as a simple arrest of development. It was not really a case of the partial modification of one sex into the other; the modifications tend to be most marked in the male because in general the female has not departed so far from the immature type. Giard's views in this connexion were no doubt mainly influenced by the account given by Pérez (1879 and 1886) of the conditions in Hymenoptera parasitized by *Stylops*, his own observations on the reduction of the endopodites of the abdominal appendages of parasitized female *Eupagurus* (see p. 77) and some very doubtfully comparable cases in the plant world (e. g. *Ustilago* and *Melandrium*), but the reduction of female abdominal appendages in parasitized brachyuran crabs was also interpreted as a modification in the male direction.

much to be said in favour of the precedent set by Wheeler (1910) of employing the word 'castration' in zoology 'in a broad sense to mean any process that interferes with or inhibits the production of ripe ova or ripe spermatozoa in the gonads of an organism and not merely in the concise original meaning as the sudden and complete extirpation of the gonads'. In this way a number of scattered but allied phenomena are brought together under one convenient heading.

The first extensive investigation of the subject was Smith's classical study of the effects of *Sacculina*, described in his Naples monograph of the *Rhizocephala* (1906) and in various subsequent papers. His researches were carried out mainly on *Inachus mauretanicus*, though other forms, such as *Eriphia spinifrons* and *Pachygrapsus marmoratus*, were also studied.

The conditions described in *Inachus* may be taken as typical of the more extreme cases of parasitic modification in Decapods. About 70 per cent. of parasitized animals show obvious modifications of the secondary sexual characters, and all show a varying degree of reduction or atrophy of the gonad. In males the copulatory styles are reduced and abdominal appendages of the adult female type may be developed, the chelae become less swollen and approximate to the more slender and flatter female type, while the abdomen becomes broadened after the fashion of the adult female. The most extreme cases of external modification are found to be associated with complete atrophy of the gonad and ducts, and even the genital pores may be obliterated. In females the abdominal appendages may be more or less reduced, but there is no effect on the chelae or abdomen, except that the latter tends to assume the adult female form at an earlier age than in uninfected animals. It was further shown that completely modified males if freed from the parasite may regenerate a hermaphrodite gonad, producing well-developed ova as well as spermatozoa.

As a result of these investigations Smith became convinced that the sexual modifications induced by parasitization were solely in the direction of male to female and never the reverse, such effects as are observable in the female consisting merely of an accelerated assumption of certain adult characters in young animals, together with minor degenerative or regressive changes in the appendages assignable to malnutrition, the changes being in no case of a specifically male kind.

In seeking for a logical hypothesis to explain these results Smith saw at once that no theory of the dependence of the secondary sexual characters on hormones produced by the gonads would cover the facts. The female secondary sexual

characters could not be determined by an ovarian hormone, for they develop in the parasitized male in the absence of any ovary, and even the idea that the mere absence of a testis conditioned the development of female characters would not meet the case.¹ He was thus led to develop his well-known theory of metabolic stimuli as the principal causes of sex differentiation, a theory whose fundamental idea is essentially in harmony with modern conclusions.²

This theory, somewhat vaguely adumbrated at first, was

¹ '... it is quite possible that the mere suppression of the testis might call forth the development of female secondary characters, but it is difficult to see how the mere suppression of the testis should make the crab subsequently develop an ovary. But even if we grant this highly improbable result, why should the suppression of the ovary in the young female crab influence the latter to assume prematurely adult female secondary characters? The explanation in fact falls to pieces when we try to apply it to the whole of the phenomena' (1913, p. 294).

² The vigour of Smith's opposition to the idea of the control of the secondary sexual characters by hormones appears to have led more than one recent critic to misunderstand his attitude and to imagine that he opposed the whole notion of hormones altogether. Salt (1927), for example, appears definitely to be under this impression and remarks (p. 307) that it is yet difficult to find any fundamental differences between Smith's views and the hormone theory which he opposed. Such observations appear to miss the essential point that it was the idea of the localized production of hormones by the gonad to which Smith objected. He constantly used the term in this restricted sense, and his objection, so far as the Arthropoda are concerned, appears to be fully justified, though its universal applicability clearly cannot now be maintained. Smith's 'sexual formative substances' might naturally be presumed to come under the general heading of internal secretions or hormones in the wider sense, and that he himself took this view is abundantly clear from his writings, as witness the following categorical statement, quoted from one of two or three passages to essentially the same effect:

'Thus the development of the secondary sexual characters is not primarily dependent on the gonad, but the development of both is dependent on a third factor. If we attempt to formulate what this factor is, it appears to me legitimate to represent it as the presence of a substance having the nature of an internal secretion, which circulates through the body and controls the differentiation of the primary and secondary characters. I have called this hypothetical substance the "sexual formative substance", and we must suppose that two kinds of it exist, male and female' (1910a, p. 599).

further developed in a series of papers between 1910 and 1914. It explained the development of the secondary sexual characters as due to the presence in the blood of male and female of sexual formative substances, which required for their manufacture two different types of metabolism. The ovary, in order to build up the yolk of the eggs, requires large amounts of fatty material, and the female sexual formative substance was conceived as being actually one of the stages in the manufacture of this material. In other words this substance was at once the essential food material or a stage in the elaboration of the essential food material of the ovary and the factor upon whose presence in a certain minimum concentration in the blood the development of the secondary sexual characters depended. In the case of the sacculinized male crabs it was suggested that the parasite extracted from the host's blood the same fatty materials as are used by the maturing ovary, and that the creation of an insistent demand for these substances resulted in the host's metabolism being altered to meet it and enable the animal to manufacture the said substances on a large scale. That is, the metabolism was altered to the female type, and this resulted in the development of female secondary sexual characters.

These views appeared to receive direct support from the demonstration by Smith (1911 and 1913) and Robson (1911) that sexually mature female crabs differ from males in having an excess of fatty material in the blood and liver, and that parasitized individuals develop a comparable excess. In parasitized specimens of both *Carcinus* (Smith) and *Inachus* (Robson) there is an excessive production of fat in the liver. In *Inachus* it also tends to flood the blood to a more or less marked extent, but this does not happen in *Carcinus*. This may perhaps be related to the fact that in *Inachus* (judging from the response produced in the secondary sexual characters) the parasite exerts a much stronger effect than in *Carcinus*. On the principle of an extra demand being met by an excessive supply, this might result in the formation of an excess of fat over and above the requirements of the parasite, while in *Carcinus* only enough is formed for the parasite's immediate needs and it is used up as fast as formed.

A comparable increase of fatty material has been shown by Kornhauser (1919) to occur in the membracid *Thelia bimaculata* parasitized by *Aphelopus theliae* (see p. 82) and by Joyet-Lavergne (1925) in the cells of the gut of the centipede *Scolopendra cingulata* parasitized by the sporozoons *Nina gracilis* and *Adelina dimidiata*, so that it appears probable that it may prove to be a very general feature of parasitic castration. Goldschmidt (1923), however, has pointed out that this fat production may be itself a secondary sexual character or, in point of fact, not the cause of the other modifications, but a coeffect with them of some less evident metabolic change. This may very well be so; but even if it is, it does not invalidate the essential part of Smith's theory. It merely relates the modification of the secondary sexual characters to more general metabolic processes instead of specifically to the fat-metabolism, an amendment which has a good deal in its favour.

Smith (1913) was able to demonstrate directly that the *Sacculina* roots do take up fat from the host's blood, and he also studied the effects of the parasite on pigment formation and on the glycogen metabolism. In parasitized animals, while the fat-producing function of the liver is stimulated, the glycogenic function is depressed. The failure of parasitized crabs to moult is apparently related to the inability to store up sufficient reserves of glycogen in the dermis to provide for the formation of new tissue which the moult involves. It is probable that in this respect also the parasitized males are more like normal females, for these do not attain so large a size as normal males, and this is probably due to a comparative poverty of glycogen reserves.

In a further theoretical consideration of the facts above outlined Smith (1913) offered a formal explanation of the mode of action of the ovary and the supposedly similar action of the *Sacculina* roots in terms of Ehrlich's side-chain theory of immunity reactions. According to this hypothesis the proteid molecules in the blood have side-chains which act as fat-links, seizing on and combining with the fat-molecules in the liver, the combined fat-links+fat-molecules then becoming detached to

float freely in the blood. The ovary or the *Sacculina* roots, as the case may be, seize on these fat molecules and liberate the fat-links to return to the liver and take up more fat. This constant removal of fat molecules from the liver leads to their regeneration in excess, and the result is a gradual flooding of the blood with fat-link+fat-combinations and free fat-links. The suggestion is made that one or both of these substances are directly concerned in conditioning the development of female secondary sexual characters, but the possibility that the latter may be related rather to other more deep-seated and less evident metabolic changes which increased fat-production entails is clearly recognized.

Potts (1906) extended the study of parasitic castration to hermit-crabs (*Eupagurus*) parasitized by another *Rhizocephalan*, *Peltogaster*, and obtained results in essential harmony with Smith's. *Eupagurus meticulosus*, which is parasitized by *Peltogaster curvatus*, was chiefly studied. In males the parasite causes the modification of the first three pairs of abdominal appendages towards the female type by enlargement of the rudimentary endopodite, which may develop the characteristic ovigerous hairs of the female. A complete series from unmodified to completely modified animals was obtained. At least a quarter of those examined were externally unaltered. The condition of the appendages in parasitized females generally recalls the young rather than the adult normal female, in contrast to what is found in *Inachus*. No real approach, however, was found to the practically uniramous male type except in three small specimens in which the reduction of the endopodite was considerable. In the second and third pairs of appendages it was only quite slightly developed; in the first it was rather larger, i.e. about half the usual size. These cases, however, were considered to be most probably really due to unusually early (possibly larval) infection, resulting in the appendages remaining in the unadvanced condition recalling those of the male, and this interpretation is supported by the very small size of the animals, as well as by the fact that the first pair of appendages, which are the earliest to develop, were more distinctly of female type than the other

two. Other evidence shows that once the full female characters are assumed they are not altered. No changes were observed after removal of the external part of the parasite, while observations on moulting and regeneration of the abdominal appendages showed that the final degree of modification is attained early, and is not altered by continued action of the parasite or its removal. The marked variability in the degree of modification in different individuals is attributed to constitutional differences.

In parasitized males ova appear in the testis while the parasite is in situ, *Eupagurus* differing in this respect from *Inachus*, where ova only develop after removal of the parasite. Potts' observations in this connexion are referred to on a previous page (68). In females the ovary merely undergoes reduction. Another point wherein *Eupagurus* differs rather strikingly from brachyuran crabs is that parasitized animals appear actually to moult more frequently under aquarium conditions than normal ones. Smith has made a suggestion to account for this difference which is discussed further on p. 89.

Potts (1910) also studied the effects of *Sacculina* on the common Shore-crab (*Carcinus moenas*). These effects, as is well known, are less considerable in *Carcinus* than in *Inachus*. The male abdomen may be broadened, but not to the extent found in the female. About a third of the crabs examined were quite unaltered. Potts did not find that the older males were not subject to modification, as asserted by Giard, nor, so far as his observations went, did he find support even for the statement that young crabs are more liable to modification than old ones.

Other external characters, e.g. the copulatory styles, are not affected, and there is no change at all in the external characters of females. The gonads are little affected. Females never bear eggs, and the ova in the ovaries remain small and deficient in yolk. In the testis a certain diminution is often noticeable, but there is never actual atrophy. There is very little reduction of the vasa deferentia and they contain ripe spermatozoa.

Guérin-Ganivet, in a mainly systematic paper on the Rhizocephala (1911), records the modifications of the hosts in the

material examined by him. Females of three species of *Eupagurus*, *Eupagurus excavatus*, *bernhardus*, and *cuanensis*, parasitized by *Peltogaster*, displayed the same conditions as in the three small females described by Potts, a supposed approach to the male type being shown in the tendency to reduce the endopodites of the abdominal appendages. The material was very scanty,¹ but the author concludes that a modification of the female in what appears to be a truly male direction is more usual than Potts supposed and invalidates Smith's generalization. A single parasitized male of *Eupagurus cuanensis* is described in which the fourth pleopod was vestigial. This is held to show that the male itself may in certain cases exhibit phenomena of regression in the abdominal appendages. The other appendages of this specimen are, however, shown by the figure to have been typically feminized.

Parasitic castration of *Galathea dispersa* by *Lernaeodiscus galathea* and of *Munida bamffica* by *Triangulus munidae* is also described, with figures, both these forms showing typical feminization of the male. In both the chelae are feminized, while the two pairs of copulatory appendages (of which No. 1 has no counterpart in the female) are merely slightly reduced, but the three pairs of pleopods of *Galathea* may be almost perfectly feminized, while those of *Munida* become intermediate, with a marked approach to the female form.

Nilsson-Cantell (1926) studied the external modifications of a large material of *Anapagurus chiroacanthus* and *Eupagurus cuanensis*, parasitized by *Peltogaster*, the latter species of crab being one of those in which Guérin-Ganivet described supposed masculinization of the female from a single example. In both species marked feminization of the male was found, without any trace of the reverse change in the female. It may therefore be considered as established that the occasional reduction of the endopodites of the appendages of

¹ One female each of *Eupagurus cuanensis* and *excavatus* and three of *Eupagurus bernhardus*, in at least two of which the modification is admitted to be trifling ('peu sensible').

parasitized female hermit-crabs¹ is due to some such exceptional circumstances as suggested by Potts; it cannot reasonably be regarded as due to any real or general masculinizing influence of the parasite.

The observations of Nilsson-Cantell show that the effects of *Peltogaster sulcatus* and *Peltogaster paguri* on *Anapagurus chiroacanthus* are not identical. The pleopods of male crabs parasitized by the former may take on a completely female character, while the special copulatory organ is little or not at all affected. On the other hand, where the parasite is *Peltogaster paguri*, the modification of the pleopods is inconsiderable, while the copulatory organ disappears. In *Eupagurus cuanensis* either species of *Peltogaster* may induce changes leading in extreme cases to complete feminization of the male. Where the parasite is attached in the immediate neighbourhood of a pleopod the whole appendage may atrophy without any differential effect on its parts. This probably explains the case of the male *Eupagurus* described by Guérin-Ganivet (see above). A detailed study of the gonads was not undertaken. Eggs were found in the testes of parasitized male *Anapagurus*, but whether these may also occur in the testes of normal males, as in *Upogebia*, is not known.

Apparently the only account of the modifications produced by Epicarids is given by Bonnier in his monograph on the Bopyridae (1900). A reduction of the liver is evidently considered very usual and the distention of the branchiostegite of the softer-shelled forms is referred to. The checking of the moult is one of the most constant effects, but is not invariable. The internal effects, though similar to those of *Rhizocephala*, are considered to be in general less accentuated. Male *Eupagurus bernhardus* parasitized by *Athelges paguri* undergo in the more highly modified examples a complete feminization of the abdominal appendages, entailing the development of the first pair, which is absent in normal males. The chelae may be

¹ It should be observed that in any case, so far as can be judged from the described cases, the reduction is never very considerable and only results in a quite imperfect approximation to the male type.

somewhat reduced and the testes are found to contain minute and imperfect spermatozoa. Male *Upogebia stellata* bearing Gyge are similarly modified in respect of the abdominal appendages, but the chelae are stated to remain as a rule larger than those of the other sex. The modifications of the appendages of *Galathea intermedia* parasitized by *Pleurocrypta* are described in more detail and figured.

Two small parasitized males are described. In both the first abdominal segment is without appendages, as in the female, the second appendage is of female type in one and in the other also of female form, but unsegmented and rather reduced. The next two pairs are in one perfectly and in the other partially feminized, while the last pair in both are only slightly modified and still much nearer the male type. The latter point is curious. If the appendages of the fifth segment developed before the rest it would be easily explicable, but this appears definitely not to be the case.

B. Insecta.

In the foregoing section, in view of the fact that the original results described in the present paper are concerned with the Crustacea, it has seemed appropriate to review the results and conclusions of other workers in this group fairly fully. In the present section the aim is slightly different and the treatment accordingly slightly less full. It is not intended to give anything approaching an exhaustive synopsis of the results obtained in the studies of parasitic castration in insects which are referred to. We are primarily concerned to indicate merely the broader conclusions, which have a bearing on the general problem of the nature of the processes at work in parasitic castration, and the facts upon which these conclusions have been based. Reference is omitted to various subsidiary points, of much interest in themselves, but more or less special to the particular forms studied; for to introduce these would carry us outside the legitimate scope of the present communication and only confuse the main issue. For more exhaustive treatment the original papers quoted should be consulted.

The two known cases in insects where parasitic castration involves a definite approximation of one sex to the other have

both been carefully studied in recent years. These two cases are those of stylopization in Hymenoptera and of the parasitization of the membracid *Thelia bimaculata* by a small polyembryonic 'wasp', *Aphelopus theliae*, of the family Dryinidae. The latter has been the subject of a most thorough and excellent study by Kornhauser (1919).

Parasitized male *Thelia* undergo a more or less marked feminization. This involves assumption of the female type of pigmentation, increase in size, modification of the abdominal sclerites and to a less extent of those of the terminal somites associated with the genital appendages. The latter organs in both sexes are reduced and tend to lose their specific characters, but each retains the general conformation characteristic of its sex. Apart from this the females are unaltered. None of the changes are due to a retention of immature characters. Internally the parasites cause varying degrees of degeneration of the gonads, but no development of oocytes occurs in the male. A definite change of metabolism in a female direction is demonstrated in the parasitized male, in which a marked increase in the accumulation of fat is observed. The normal male is characterized by high metabolism and high oxidation rate. The females develop more slowly and grow larger, and they actually make their appearance later in the season than the males. Parasitized males, on the other hand, appear at the same time as the normal females, showing that their development has been retarded. They have actually become animals of high storage capacity like the females, as is shown by the increased deposition of fat. Two particularly noteworthy individuals are described, namely, a parasitized male markedly feminized externally, but with a normal unreduced testis, and an unparasitized nymph with a typical female soma and male gonads. These two cases illustrate clearly the independence of soma and gonad. Mainly on the ground of the natural and experimental evidence of such independence in insects Kornhauser rejects Smith's hypothesis of a demand coming from the ovary affecting the metabolism of the somatic cells and the parasite inducing its effect by acting in a similar way. It is suggested that recently evolved characters are the most liable to be affected. Reasons are given for

regarding the female *Thelia* as more conservative in respect of its external characters than the male. 'The extragenital sexual characteristics may be due to genes which in many cases modify characteristics once common to both sexes, but now exhibited in a primitive condition only in the more stable female. The metabolism of the parasites, altering the constitution of the host's haemolymph which bathes the developing cells, may offer an environment unsuitable for these recent genes to find their expression, and leave the male individual in a more primitive state, exhibiting characteristics now found in the female.'

The term Stylopization is applied to the parasitization of solitary bees and wasps by the peculiar insects *Stylops* and *Xenos*, constituting the order Strepsiptera. The effects of these organisms on their hosts were described by Pérez as long ago as 1886. Since then various writers have contributed observations on the subject, but for present purposes it is only necessary to notice three papers, namely, the recent careful study of Salt (1927), who summarizes the results of previous workers, and the earlier contributions of Wheeler (1910) and of Smith and Hamm (1914).

The Strepsiptera are extraordinary insects. The following brief outline of their life-history is condensed from Salt: The degenerate female, which retains a larval form, is embedded in the abdomen of the host, only the chitinized cephalothorax protruding between the abdominal sclerites. The eggs develop in the body-cavity and become larvae, which escape to the exterior by the so-called 'brood-canal' which runs forward to open anteriorly. They then scatter over the body of the host and eventually, probably by getting on to flowers visited by both stylopized and healthy insects, contrive to attach themselves to another bee or wasp of the same species and so get transferred to the nest, where they attack the larvae, in the bodies of which they develop. The female remains in the host's abdomen, as already described, absorbing nutriment from the blood, but the winged male emerges in due course to pursue a brief free-living existence, during which the female is fertilized through the brood-canal.

The most noteworthy feature of stylopization is that external modifications of the female in what appears to be a specifically male direction occur, as well as the converse. The most striking instance of an interchange of characters between male and female is found in connexion with the colour of the clypeus in the bees of the genus *Andrena*, in some species of which this region is yellow in the male and black in the female. As a result of stylopization the female may assume a yellow coloration as in the male, while in the male the yellow tends to disappear and to be replaced by black. These changes were described by Pérez, together with others of an apparently similar nature, though less striking. For example, stylopization in females causes a narrowing of the tibia and basitarsus of the last pair of legs and a reduction of their pollen-collecting apparatus of special hairs, resulting in a close approach to the male condition. The reversed changes in the male also occur, but appear to be rarer, and in both sexes much individual variation in the degree of modification is found. The facial foveae, depressions on either side of the face, which occur in the female, but are reduced or absent in the male, are reduced in parasitized females and may occur in a reduced form in males of species in which they are normally absent in that sex. Various other changes occur which are common to both sexes and affect specific characters, giving parasitized animals an illusory appearance of being distinct species. The alterations in the secondary sexual characters are regarded by Pérez as true inversions, the female taking on male attributes and the male female ones.

Wheeler (1910) examined a large number of specimens of the wasp *Polistes* parasitized by *Xenos* and failed to find any modifications of a morphological character which could be definitely attributed to the presence of the parasites. His paper is particularly valuable on account of the excellent review and discussion which it contains of all the then available data on the effects of castration in Arthropods and more particularly in insects, the term 'castration' being used in its broadest sense as defined in the foot-note on p. 72, thus including parasitic castration, under which heading reference is made to the many

curious and interesting cases which do not entail any actual inversion of the sex characters and which are not considered in the present paper.

The remarkable changes described by Pérez in stylized *Andrena* were naturally not overlooked by Geoffrey Smith, since they appeared to constitute a difficulty in the way of the general application of his theory, and almost his last scientific paper, published jointly with A. H. Hamm (1914), was concerned with this subject. The modifications in the species which Smith and Hamm were able to examine were for the most part rather less pronounced than those described by Pérez, but in *Andrena chrysosceles* they observed the masculinizing of the female clypeus, which other workers since Pérez had apparently not noted. The effects of the Strepsiptera, which are much slighter than those of Rhizocephala, are ascribed to 'a merely quantitative abstraction of nutriment from the gonad, leading to its partial atrophy, and not to a qualitative alteration such as is brought about by *Sacculina*'. With regard to the acquirement of the yellow clypeus by stylized females it is suggested that this is not due to a specific masculinizing influence of the parasite, but is a direct consequence of ovarian atrophy, the ovary being supposed to exert an inhibiting influence on the development of yellow pigment in an analogous manner to the inhibiting of male plumage characters by the ovary in birds.

The most recent and exhaustive account of the effects of stylization is that of Salt (1927), who, as already stated, summarizes the results and conclusions of previous workers in addition to his own. His paper should be consulted for a complete bibliography and references to the observations of Pierce, Perkins, and many others of minor importance which are not specifically referred to here. Most observers since Pérez have tended to regard the changes, apart from the indisputable case of the colour of the clypeus, as of a purely negative kind, a sort of 'convergence toward a mean condition, each sex losing some of its own peculiar attributes', but Salt draws attention to various positive inversions of external sex characters. These include some new instances of a definite interchange—e.g. in respect of the length of the antennal segments in *Andrena*

hirticineta and in the acquisition by the females of *Andrena canadensis* of the characteristic angular form of the cheek of the male and the loss of this character by males. The interchange of sexual characters of the clypeus is shown to occur also in wasps of the genera *Odynerus* and *Ancistrocerus*. In the fossorial wasps of the genus *Sphex* stylopization produces changes in the amount of the pubescence on the head and thorax, parasitized females of *Sphex pictipennis* showing an approach to the male type in the increased pilosity of these regions, a positive change. Negative changes are seen in the tendency of parasitized males to lose a characteristic patch of gold or silvery hairs at the tip of the propodeum and of females to lose the special digging spines on the legs.

The internal effects of stylopization are much less marked than in the cases of parasitization of Crustacea by *Rhizocephala* and *Epicarida* and are confined to a mere reduction of the organs without any modification of either sex towards the other. Females are generally if not always rendered sterile, but males generally continue to produce ripe spermatozoa. It is emphasized that animals affected by parasitic castration may be regarded as intersexes, and it is suggested that the extent to which characters are affected is related to the time of their development in ontogeny (cf. Goldschmidt's results on intersexes in *Lymantria*). Smith's hypothesis that the parasite acts specifically as an ovary is held to be untenable, but his general theory of metabolic stimulation is considered to be fundamentally in accord with Goldschmidt's theory of sex. The final conclusion reached is that 'the effect of stylopization is to upset the nutritional balance of the host, which affects the reaction of the sexual hormones and produces intersexes'.

There has now been placed before the reader the whole of the material upon which, in the present state of knowledge, any attempt at a general explanation of the phenomena of parasitic castration must be based. In the following section the conflicting conclusions noted above will be more fully discussed.

VI. DISCUSSION.

The present account represents to the best of the writer's belief the first attempt at a general and fairly complete study of the effects of an Epicaridan isopod on its host, and although more work is required upon certain points, as, for example, upon the question of what happens to the gonad if the parasite is lost, the material provided in the foregoing pages is sufficient to enable a comparison with other comparable cases to be undertaken with advantage. It will be seen from the preceding review that there are now three instances of parasitic castration in Crustacea which have been fairly fully studied, namely, those of *Inachus* (and other *Brachyura*) and *Sacculina*, Hermit-crabs and *Peltogaster*, and the present case of *Upogebia* and *Gyge*. The case of the Epicarid is interesting amongst other reasons, because it affords an example of a completely external parasite producing modifications no less extensive than those caused by the essentially internal parasites previously studied. It is true that in some respects the effects are not so accentuated as in the case of *Inachus* and *Sacculina* (e.g. growth and moulting are not prevented, the activity and viability of the animals are not seriously affected, the genital pores are apparently never obliterated, sperm may often go on being produced, and so on), yet the constant development of female first abdominal appendages in the male, the effects on the chelae, and the frequent complete obliteration of the gonad are as striking as anything found in hosts parasitized by *Rhizocephala*, and in fact are very much more pronounced than are the effects of *Sacculina* upon such crabs as *Carcinus*. This emphasizes in a very convincing manner the conclusion, already firmly established, that the changes observed are dependent on an effect on the general metabolism and not on any direct lesion of the gonad. Even Geoffrey Smith, who insisted upon this fact most strongly, was apparently inclined to assume that *Sacculina* with its far-reaching system of absorptive roots must necessarily exert a more drastic action than the other types of parasite which are known to produce parasitic castration. In comparing the phenomena of stylopiza-

tion and sacculinization he suggested (1914) that the less radical effects of the former were to be accounted for by supposing that the Stylops by simply absorbing the host's blood effected a 'merely quantitative abstraction of nutriment normally destined for the reproductive glands', leading to simple atrophy of the gonad and reduction of the secondary sexual characters, whereas the roots of *Rhizocephala* exercised a selective action, extracting from the blood a particular type of nutriment—e. g. fat—in such a way as to divert the metabolism of the host to the female state.

In view, however, of the far-reaching effects of simple blood-suckers like the Bopyridae on the one hand and of the considerable effects of *Sacculina* on some species of crab on the other, it does not seem that this cleavage between the *Sacculina* type and the other can be maintained. It is quite easily understandable that a parasite simply taking up blood as it comes, but taking it in considerable quantities, might effect, through the resultant drain upon those blood constituents which are most costly for the organism to prepare, a modification of metabolism no less radical than that caused by one exercising a directly selective action on the nutrient materials. On the other hand, another form feeding in a comparable manner, but taking up less blood or blood of a different and relatively less expensive composition, or acting on a less sensitively constituted host, might check the latter's development in some respects without effecting any radical metabolic change.

A point of importance in connexion with actual fixation on the host wherein *Gyge* is found to differ from *Sacculina* and *Peltogaster* is that this takes place only (or with only the rarest exceptions) during a relatively short period of the very early life of the host. The consequence is that whereas in crabs parasitized by *Sacculina* a quite considerable number, of the order of about a quarter to a third of the total, are externally unmodified, having apparently been attacked too late in life, such a thing is unknown amongst any but the very smallest individuals of *Upogebia* parasitized by *Gyge*. In the latter case the animal grows up under the parasite's influ-

ence, and hence it is possible to study the effects without the confusing element introduced where parasitization can occur at widely different ages. Yet we have seen that even parasitization at different points within the comparatively small range between about 3 and 6 mm. carapace length may result in quite marked differences in the consequences to the sexual organs, for the variability observed is considerable. But it is impossible to escape the conclusion, already emphasized, that this variability cannot be dependent solely upon the time of parasitization, but must be due also to constitutional differences in the animals. The same conclusion is insisted upon by Potts (1906) with reference to *Eupagurus* and *Peltogaster*, and it suggests a field for future work by the invertebrate physiologist and biochemist.

The fact that a pronounced degree of modification is not incompatible with extensive growth appears to be now positively demonstrated for the first time in parasitized Crustacea, though it is paralleled by Kornhauser's findings (1919) in the case of *Thelia*, where parasitized males grow larger than normal ones. In *Inachus Sacculina* checks growth and moulting completely, and Smith (1913) has shown that this is probably related to a demonstrable inhibition of the production of glycogen, the main reserve material for formation of new tissue. In *Eupagurus Peltogaster* appears actually to increase the frequency of moults (Potts, 1906), but there is some evidence that it nevertheless reduces the amount of growth. Smith (1913) has suggested that *Peltogaster* may inhibit or retard growth by reducing the glycogen supply like *Sacculina*, and yet not interfere with the actual process of moulting, so that this occurs without significant increase in size. This suggestion appears to receive some support from the fact that infected hermit-crabs do not average larger than normal ones, as they would be expected to do if the more frequent moults were each accompanied by an increase of size of the same order as occurs in uninfected animals. But, whatever may be the case in *Eupagurus*, it is quite certain that in *Upogebia* parasitized by *Gyge* a large amount of growth does take place, for although fixation occurs while the host is

very small, parasitized animals grow just as large as others. That they may take longer to reach a given size than uninfected animals is possible, though quite doubtful; but it does not in any case affect the main point that the total increment in size is not diminished. It is clear, therefore, that the inhibition of growth must be regarded as an incidental and not a necessary feature of parasitic castration,¹ dependent upon particular physiological conditions which are not of universal application. A study of the glycogen metabolism in normal and parasitized *Eupagurus* and *Upogebia* is desirable for the purpose of comparison with *Inachus*.

With reference to the development of egg-cells in the testes of parasitized males, the three crustacean types available for comparison show in a rather interesting manner three different conditions with respect to these. In *Inachus* they develop only when the host is freed from the parasite, in *Eupagurus* they appear in the testes while the parasite is *in situ*, while in *Upogebia littoralis* they occur on a small scale in the testes of many normal animals, and the tendency to produce them is accentuated by parasitization. The production of a small number of scattered oocytes in the testes of otherwise normal males is a peculiarity not confined to *Upogebia* amongst the Decapods. It would appear to be not very uncommon in *Potamobius* (*Astacus*)² and has also been described in *Homarus*.³ Though the significance of the facts is not altogether clear, it is of interest to recall that in at least two other species of *Upogebia* (*Upogebia major* and *stellata*) the production of these cells appears to have become constant and localized in a special region of the testis not represented in *Upogebia littoralis*. In *Upogebia*

¹ Potts (1910) has given reasons for supposing that the prevention of moulting in parasitized crabs is purely mechanical, the *Sacculina* acting as a rivet which prevents the abdomen being withdrawn from its chitinous encasement, but it seems clear that without some inhibition of a more positive kind than this an abortive attempt at moulting would take place, with resultant death of both crab and parasite. Smith (1913, p. 279) has made a substantially similar objection.

² La Valette St. George (1892), Garnier (1901), Prowazek (1902).

³ Herrmann (1890).

stellata this condition may be associated with the presence of rudimentary oviducts and corresponding supernumerary genital pores. This last case seems to link up the above-mentioned instances of partial and non-functional hermaphroditism of the gonad with those other intersexual types, occurring in some Decapods, which may involve the presence not only of supernumerary ducts and apertures but of the external appendages and other secondary sexual characters of the opposite sex (see p. 108). Whether these two sets of phenomena are really directly related is, however, open to question, for in the latter cases, so far as can be judged from the literature, the gonad is more often than not a normal ovary or testis, showing no signs of hermaphroditism.

The production of egg-cells in the testes of crustaceans and other animals is an occurrence provocative of reflection on the whole nature and mode of operation of the sex-determining processes. It can be confidently asserted as a result of modern work that sex is mainly, if not wholly, a question of rates and intensities of metabolic processes. In cases like those under discussion we see how such differences from the type to which the individual as a whole belongs may be localized in a single cell or in a few isolated cells scattered in the testis. And we see how the influence of a parasite on the same animal can cause the same difference to assert itself in a larger number of cells than before or even in a whole tract of gonad, while other regions of the organ can go on functioning in the normal way. What is the precise nature and mode of action of processes which can operate in this curiously localized manner? Is it a question, here, as elsewhere, of chromosomal differences in the cells, and if so, in what way does parasitization encourage aberrations of chromosome distribution in favour of the female type? Or are we not dealing with chromosome differences at all in these cases? We cannot at present answer such questions.

It is not necessary, however, to wait for a complete elucidation of these problems before attempting to arrive at some general conclusions as to the nature of the processes involved in parasitic castration and their relation to the normal sex-determining processes. Geoffrey Smith's conclusions have been

sketched in the previous section, but in view of the work which has been done since his time, especially on parasitic castration in insects, and the criticisms which have been made of his hypothesis, a general reconsideration of the position seems desirable at the present time, and to this we may now proceed.

At the outset it is necessary to insist that the alteration of secondary sexual characters is a real inversion. The notion seems still to be not altogether dead that the feminization of the male found in cases of parasitic castration is not really a case of development of specifically female characteristics at all, but of a return to or retention of characters wherein the female has departed less widely from the ancestral type than the male. This idea is as old as the discovery of parasitic castration itself, for it was entertained by Giard. The present writer would have supposed that it had been sufficiently exploded already, but it still crops up. It forms the basis of Kornhauser's attempted explanation of the modifications of *Thelia* (1919) and I have heard it expressed by others. I fail to see how any one fairly considering all the facts can seriously maintain such a view. The contention that the alleged modifications were really retentions of juvenile features or 'reversions' to an 'undifferentiated ancestral condition' was combated by Smith (1910 *a* and *b*), and his arguments are equally cogent to-day. The facts which he emphasized, that in parasitized *Inachus* the types of abdomen and appendages assumed are of the characteristic adult female form and quite different from the less differentiated juvenile type, and that on recovery from parasitization a certain number of males develop well-yolked ova are quite inconsistent with any such explanations. Indeed, it appears to me that any one carefully reading Smith's original papers will find this conclusion so incontestably demonstrated for the forms with which he was primarily concerned that I shall confine myself here to certain points wherein my own results have a bearing on the subject.

In the matter, then, of chela growth in *Upogebia* it appears to me impossible logically to resist the conclusion that the mode of growth of the chela in parasitized males is specifically and definitely feminized. There appears to be no sort of

justification for imagining that the mode of growth of the female is of a less specialized kind than that of the male. The most reasonable view appears to be that the male and female modes of growth are equally characteristic and specific. The generalized type of chela growth would presumably be one uniform in rate with the rest of the body. It is true that in the latter part of life the parasitized male chela does appear from the measurements to tend to grow in breadth at a rate more nearly uniform with the carapace than in the normal female, but even so it remains very close to the female type and widely divergent from the normal male, and I submit that the small difference from the normal female is adequately explained by the suggestion I have offered (p. 29). Any one who seeks to attach any weight to the small difference as evidence against my view has, moreover, to explain away the complete agreement found between the parasitized male and normal female curves in respect of chela length.

Again, it might be argued that the scattered egg-cells which occur in the testes of *Upogebia* and other forms are not really egg-cells, but hypertrophied spermatogonia or spermatocytes simulating egg-cells. But when we find in parasitized males palpably similar cells increasing in size and actually forming yolk¹ in some cases, such a view can no longer reasonably be maintained. Further, it is important to observe that in different, but allied, forms parasitization may cause in one case the loss and in another the development of homologous organs. Thus, in *Upogebia*, where only the female normally possesses appendages on the first abdominal segment, the parasitized male develops these, while in *Galathea*, where only the male normally possesses them, the parasitized male loses them. It cannot be said that the presence of these appendages is primitive in *Upogebia* and their absence primitive in *Galathea*. The only possible way of logically explaining the observed

¹ Yolk-formation in the essentially similar cells occurring in the testes of *Potamobius* is described by La Valette St. George (1892) and others, apart from any parasitic influence, and according to the observations of Runnström it may also occur in the testes of apparently unparasitized individuals of *Upogebia stellata* (cf. p. 52).

reaction of these two forms is to admit that in both cases the male assumes the characters peculiar to the female of its species.

We come, then, to this point in the argument, that parasitic castration in the cases under consideration results in a positive and unequivocal inversion of the sex characters, and we may go a step farther and add that the conditions now described for *Upogebia* entirely support Smith's contention that the inversion is not reciprocal, but affects the male sex only. Thus, so far at least as the Crustacea are concerned, Smith's original hypothesis that the parasite acts on the host like an adult ovary still appears to cover the facts. Whether it can be accepted as a really valid explanation depends on whether it can be satisfactorily applied to the cases of parasitic castration in insects which have been subsequently investigated. We have seen in the previous section that in point of fact both Kornhauser (1919) and Salt (1927) reject Smith's explanation, and we have seen some of their reasons. These reasons will be considered more fully in a moment, but before doing this it is permissible to take a glance at the alternatives which they have to offer. Kornhauser believes that it is the phylogenetically newer characters which are affected. Undoubtedly there is a certain element of truth in this: manifestly the characters altered are not the more fundamental ones of ordinal, family, or even generic value, but this is merely an incidental fact throwing no real light on the nature of the processes at work and certainly not explaining why the modifications should involve an approximation of one sex towards the other. We have seen that the explanation which Kornhauser offers for this latter fact in the particular case of *Thelia*, that the male is more modified from the primitive type than the female, cannot possibly hold good as a general explanation.

Salt concludes that the parasites 'upset the nutritional balance of the host, which affects the reaction of the sexual hormones and produces intersexes'. In an elaborate discussion he insists on the essential unity of the phenomena of intersexuality, as elucidated by the well-known work of Goldschmidt and others, and of parasitic castration. With this conclusion

I quite agree, but it is really nothing new. Caullery (1922) appears to have been one of the first, if not the first, to insist upon it; Goldschmidt himself (1923) treats parasitic castration as a special category of intersexuality; and indeed the validity of the conception appears to be now widely recognized. Clearly the cases under discussion fall within the definition of intersexes as individuals which have developed up to a certain point as either male or female and then continued their development along the lines of the other sex.¹ I think it necessary to remark, however, that it would be altogether premature and probably wrong to assume too close an agreement between the processes involved in parasitic castration and in the zygotic intersexuality of forms like *Lymantria*. Caution is necessary here: it must be remembered that several distinct types of intersexuality are already known.

But if we admit that parasitically modified animals are properly regarded as a type of intersex we are still not much nearer understanding how the changes are brought about. Salt's conclusion that they are accomplished by upsetting the nutritional balance of the organism is no doubt true so far as it goes, but this is not very far. We are left, in fact, with Smith's suggestion as the only one which makes any approach to a real explanation of the way in which the parasite exerts its effect, and it is desirable therefore to inquire whether Kornhauser and

¹ Salt suggests that the variability in the effects of stylopization can be explained in a similar manner to the different grades of intersexes in *Lymantria*, that is to say, that structures which differentiate latest in development are the first to be affected. He believes that the observed variability is thus dependent primarily on the time of parasitization, in conjunction with some minor factors, such as the number of parasites present, the sex of the parasite (the male *Stylops* generally exerting a greater influence than the female), and so on. This may well be so, but Salt does not appear to consider the possibility of individual variation in the degree of responsiveness as a possible further factor. Whatever may be the case in stylopized Hymenoptera there can be no reasonable doubt that such variability exists in *Upogebia*, as I have already insisted. The fact that a certain modification or a given degree of a certain modification may exist in different individuals in conjunction with entirely different degrees of modification in respect of a second character can hardly be explained in any other way.

Salt have really good reasons for rejecting it. It is clearly dependent at the outset on the correctness of the conclusion that the parasites never cause modification of the female type towards the male, but only of the male towards the female. The critical case in this connexion is that of *Stylops*. Salt, being not unnaturally impressed by the actual exchange of some secondary sexual characters in stylopized bees and wasps, implies in his discussion that Smith was led to over-emphasize the one-sidedness of the modifications in sacculinized Crustacea, and appears to suggest that in these also there is a tendency, though faint, to develop male characters in the female. He points out that in *Inachus*, as recorded by Smith, 'the infected females have swimmerets reduced or rudimentary' and cites from Potts's work the 'suggestion that female *Eupagurus* infected by *Peltogaster* may assume male characters', though we have seen in the previous section that this 'suggestion' is almost certainly to be explained in another way (see pp. 77 and 79-80). Further, he adduces the fact that female intersexes are definitely known to occur in Crustacea in support of his view. He goes on to say (p. 312), 'If *Sacculina* acts specifically as an ovary in developing the female sexual formative substance and producing adult female characteristics, why should the swimmerets of adult females be rudimentary? If *Sacculina* acts only as an ovary and the action of *Stylops* on *Andrena* is at all similar (as most probably it is), then a *Stylops* must act in a male bee specifically as an ovary, but in the female as a testis. To account for these cases of female intersexes in Crustacea, let a male as well as a female sexual formative substance be produced, make the necessary modifications in his hypothesis to accommodate the interaction of the two, and Smith's theory of metabolic stimulation will differ little or nothing from the explanation we have offered for the same phenomena based on Goldschmidt's theory of intersexuality.'

Now Smith himself clearly recognized the probability of there being a male as well as a female sexual formative substance,¹

¹ Cf. for example, the passage in the quotation given in the foot-note on p. 74: 'I have called this hypothetical substance the "sexual formative

and the appropriateness of treating parasitic castration as a form of intersexuality has been admitted, while we may also readily concede that there is no fundamental discrepancy between Goldschmidt's theory of sex and Smith's, but I am quite unable to agree with the implication of the earlier sentences of the passage quoted. So far, at least, as the Crustacea are concerned I wish to insist most strongly on the validity of Smith's conclusion as to the exclusively feminizing influence of the parasites. My own results are completely in accordance with his. In *Upogebia* I have described a striking feminization of the male and nothing even suggesting masculinization of the female. The slight reduction of the chelae in parasitized females is perfectly analogous to the reduction of the swimmerets in parasitized female crabs, but if the modifications really indicated a tendency in the male direction the chelae would have to be somewhat enlarged instead of somewhat reduced. The complete absence of a tendency for parasitized female *Upogebia* to be modified towards the male type in any character is, I think, sufficiently evident from the foregoing pages, and it is unnecessary to labour the point.

The reduction of the swimmerets in parasitized female crabs really presents no difficulty at all. It is not supposed that the effect of the parasite is perfectly identical with that of an ovary. All that is suggested is that the demands on the organism made by the parasite are so far similar to those made by the ovary as to induce a state of metabolism sufficiently in agreement with that of the female as to condition the development of certain female characters. Qualitatively the similarity of the effects might amount to practical identity or it might not; but quantitatively there is certainly a difference. It is clear that in some respects the demands of the parasite are more exhausting than those of the ovary, for in the case of *Sacculina* it checks growth and moulting, which palpably the normal ovary does not. The failure of certain appendages to develop quite so far or to quite the same size as in normal females is thus a perfectly natural retardation consequent upon substance", and we must suppose that two kinds of it exist, male and female.'

the drain on the host's resources caused by the parasite and no more.

The argument based by Salt on the fact that female intersexes do occur in Crustacea is not really relevant to the subject under discussion. Because parasitic castration appears to be a form of intersexuality we are not entitled to argue that what is true with reference to intersexes arising in other ways must be true also in the case of parasitic castration. And in any case, as Goldschmidt himself observes (1923, pp. 98 and 99), we do not even know the nature of crustacean intersexes with any certainty. I venture, then, to reassert most emphatically the conclusion that the changes found in the parasitized Crustacea which have so far been studied, in so far as they involve the approximation of one sex towards the other, are exclusively in the direction of feminization.

What, then, is to be said of the assumption of apparently specifically male characters by females in stylopized bees and wasps? We have seen that this apparent anomaly was not overlooked by Smith. In order to account for it he suggested (1914) that the assumption by the parasitized female of certain characters normally associated with the male was not due to any specifically masculinizing influence of the parasite, but could be explained by supposing that these characters tended to develop in all individuals except in so far as they were inhibited by the ovary. According to this idea the development of the said characters was simply a consequence of the damage to the ovary, analogous to the development of male plumage in female birds. It is evident that this explanation cannot now be maintained, since it has been shown that the so-called secondary sexual characters of insects are independent of the gonads. Yet the underlying idea that the development of these apparently male characters is not due to a specific action of the parasite may still be sound.

It appears entirely possible that the appearance of these characters in parasitized females may in fact be due to their development being regulated by some peculiar conditions in Hymenoptera not paralleled in the other forms. It is already

known from work on the bee and some other members of the group that the methods of sex determination in Hymenoptera are distinctly peculiar. We find in this group, for example, the highly unusual arrangement of having twice as many chromosomes in the female as in the male. In the gametogenesis of the male the reduction division is omitted and so the spermatozoa have the same number of chromosomes as the male somatic cells. The ova undergo the maturation divisions in the ordinary way and thus have the same reduced number of chromosomes as the male gametes. An egg, then, which is fertilized becomes a female and one which is not fertilized becomes a male. These things in themselves show that a distinctly unusual state of affairs exists in these insects. But there is evidence, that in certain circumstances drones may arise from fertilized eggs (Bresslau (1908) and others). The evidence, which is considerable and not to be lightly rejected, is favourably considered by Goldschmidt (1923, p. 197), who gives fuller references and concludes that if it is correct the result must be achieved by special feeding (cf. the nutritional control of the development of queens or workers). In other words it would appear that the workers may have 'the power of increasing the velocity of reaction of the male enzyme, or what is more probable . . . of disturbing the velocity of differentiation of the organs, or even of altering directly the chemical reactions of the body (by means of the special nature of the food) and thus bringing forth male bees out of fertilized and therefore female eggs'. If this kind of thing can occur it would be by no means surprising to find that the nutritional disturbance resulting from parasitization in these forms may result in the appearance of certain male characters in females without the parasite exerting any directly masculinizing influence. I am aware that my omission to formulate a more precise explanation may be seized upon as a weak point in the argument; but where obviously much more remains to be learnt about the control of the sex characters in this group I do not think one can reasonably be expected to do more than indicate the general lines along which a solution may be sought. At any rate, after the most careful consideration of the facts, I am convinced that the evidence from all other sources is so

consistently against any specifically masculinizing influence being exerted by parasites, in the sense that a specifically feminizing influence is exerted by them, that I feel certain that its apparent occurrence in stylopization must be explained in some special way. There is no denying that the phenomenon is highly exceptional, and probably unique amongst the known instances of parasitic castration. It has been sufficiently emphasized already that the cases in Crustacea all agree in that wherever there is a clear change of one sex towards the other this is exclusively in the female direction, and even in other insects there is no evidence of masculinization, for Kornhauser found only modifications in the female direction in *Thelia* parasitized by *Aphelopus*. The difficulty of accounting for the exchange of characters between stylopized males and females is not lessened even if Salt's views are adopted, for it is not at all clear from his discussion why on his theory parasitization should modify each sex in the direction of its opposite, instead of favouring one type of metabolism against the other. We conclude, then, that even though a complete explanation has not yet been formulated, the development of certain male characters by stylopized female bees and wasps is not directly due to the action of the parasite.

It may be added that even in the improbable event (as I consider it) of a specific masculinizing influence of the Strepsiptera being proved, there would be no justification for assuming in defiance of the evidence that a similar masculinization must necessarily occur in Crustacea parasitized by Rhizocephala and Epicarida. Considerable caution must be observed in arguing from the Insecta to the Crustacea or the Crustacea to the Insecta. Although both groups belong to the Arthropoda it cannot be safely assumed that the conditions are necessarily the same in both. The differences in the mode of determination of the sex characters in birds and mammals amongst vertebrates is sufficient warning against such an assumption.

If the exclusively feminizing action of the parasites in parasitic castration is admitted, an alleged difficulty in the way of Smith's theory has been dismissed. It remains to consider whether apart from this his explanation is acceptable or not.

It may be said at once that although a good deal more work, especially on the physiological and biochemical side, is needed before the fundamental processes involved will be fully understood, and although some details of Smith's hypothesis may be open to criticism, yet the general conception of the parasite affecting the metabolism in a similar manner to an adult ovary appears still to provide the most consistent and satisfactory way of accounting for the facts. Smith's and Robson's results on the fat metabolism in normal and sacculinized crabs and Kornhauser's work on *Thelia* parasitized by *Aphelopus* provide actual proof that a change of metabolism towards the female type does occur in the parasitized male, even though the actual increase of fat should prove to be one of the effects of the change and not itself the cause of the other somatic modifications. Perhaps an even more definite piece of evidence that the parasite acts like an adult ovary is the observation of Smith, already emphasized, that *Sacculina* causes young female crabs to assume the adult female type of abdomen prematurely.

It will be observed that the two writers who reject this view were both working on insects, and undoubtedly both attached great weight to the fact that the experimental evidence in insects appears to be strongly against any such influence of the ovary on the soma as Smith postulated. The work of Oudemans (1898), Kellogg (1904), Meisenheimer (1909), Regen (1909-10), Kopeć (1911 and 1913), and others has shown clearly that the so-called secondary sexual characters of insects are not altered by removal of the gonads and in-grafting of those of the opposite sex, even when these operations are performed at an early age. Each cell normally pursues its differentiation by virtue of its own chromosome complex. Clearly these facts seem at first sight difficult to reconcile with Smith's conclusion, but before considering this point further it will be well to stop for a moment to consider whether the difficulty is likely to apply to the Crustacea or whether it is one peculiar to the insects only. It has already been observed that there is no *a priori* reason why the conditions in the Insecta and the Crustacea should be the same.

Most of the text-books concerned with sex-determination and

allied matters are a good deal more reticent about Crustacea than about insects. Certainly a good deal less is known about the former, but more is known than is apt to appear, so that a brief consideration of the subject here may serve a useful purpose in more ways than one. There is no direct experimental evidence as to how the sex characters are determined in the Crustacea. No one, so far as I am aware, has yet succeeded in performing the crucial experiments of removal or transplantation of gonads, an operation evidently even more difficult than in insects owing to the diffuseness of the organs and the readiness with which the animals succumb to injury of the carapace. It is not, however, necessary to look very far in order to find evidence that the secondary sexual characters must be at least in a very large measure independent of the gonad. Even the observations recorded in this paper and the similar ones made in other cases of parasitic castration in Crustacea make it clear that there must be a considerable degree of such independence, and this is made even more evident by some of the abnormalities which occur apart from parasitization. For present purposes we can confine our attention to those occurring in the Decapoda, to which group all the known forms of Crustacea subject to parasitic castration belong.

One of the clearest pieces of evidence is provided by the fact that true gynandromorphs occur in the Decapoda. They seem to be rarer than in insects and are much less frequently referred to in the literature, but a perfect bilateral gynandromorph lobster was described by Nicholls in the Philosophical Transactions of the Royal Society as long ago as 1780, and more recently Bürger (1902) has described and figured an example of *Palimnurus frontalis* which appears to have been a complete bilateral gynandromorph externally, though unfortunately the structure of the internal organs is not known..

These cases show that the somatic characters of one sex can be differentiated perfectly in the presence of a gonad of the other sex and that there is no overriding hormonal or other influence, though they do not entirely rule out all effects of the gonad on the soma, as witness the case of birds, in which such an effect is indisputable and yet gynandromorphs occur.

The same conclusion, that the development of the external sex-characters and of the gonad are at least in the main independent of one another, is indicated by the various intersexual forms which have been described. These abnormalities involve the development in varying degrees in individuals of one sex or the other of genital pores, ducts, and secondary sexual characters of the opposite sex. Supernumerary genital apertures have been described in so many forms that it is unnecessary to give references to individual cases. Many of them have been referred to by Ridewood (1909). Such supernumerary apertures definitely associated with ducts have been described in *Homarus* (Ridewood, 1909), *Potamobius* (Desmarest, 1848; Benham, 1891; Bateson, 1894, and others), and *Parastacus hassleri* (Lönnerberg, 1898).¹ Comparable conditions in the genus *Upogebia* have been discussed in an earlier section (p. 51). The accessory ducts sometimes look like mere reduplications of the normal pair, but in other cases resemble those of the opposite sex more or less closely. Cases in which the appendages and other secondary sexual characters are involved occur in *Potamobius* and *Cambarus* and no doubt in other forms too. In *Cambarus* they seem to be particularly frequent and many instances have been described by Faxon (1885, 1890, 1898), Hay (1905), and Turner (1924, 1925 *a* and *b*), showing all sorts of admixtures of sex characters.² Hay records

¹ In this form the development in each sex of supernumerary ducts and apertures corresponding to those of the opposite sex appears to have become constant, and there is some evidence that egg-cells are developed in the testis. Such a condition of partial and evidently non-functional hermaphroditism might be regarded as leading on to the functional hermaphrodite condition found in the Mediterranean prawn, *Lysemata seticaudata* (Spitschakoff, 1912) and the Thalassinid *Calocaris macandreae* already referred to (Runnström, 1925).

² It is noteworthy that the large majority of these intersexual *Cambarus* are female intersexes—i. e. females more or less modified in a male direction—though male intersexes also occur. This is also true of the other cases referred to above. The fact that female intersexes are thus markedly more frequent in the Decapoda than male ones is significant in connexion with parasitic castration. Modifications of the female in a male direction are evidently in general more readily produced in this group than the reverse, yet in the known cases of parasitic castration in Decapods not a

two notable cases: (1) predominantly male in external appearance (though with female pores and annulus ventralis), but gonad a typical ovary with nearly mature eggs, and (2) completely male externally, but having a large ovary full of almost ripe eggs, associated with a minute testis and sperm-duct on one side. The reversal of sex characters in soma and gonad is not here quite so perfect as in some insect cases, but indicates at least a high degree of independence.

To sum up, we must conclude that the differentiation of the so-called secondary sexual characters in Decapod Crustacea is, at least in the main, independent of the gonad. There is no conclusive evidence of any influence of the ovary on the soma and some evidence against it. An experimental disproof of such an influence in normal development is lacking, but the facts so far as they go are in harmony with what is known about the Insecta, where such an experimental disproof has been obtained. It may be conceded, therefore, that it is reasonable to suppose that the relations of gonad and soma in the two classes are similar, though it would not have been permissible to assume this *a priori*.

The position, then, is that an influence of the ovary on the somatic characters in normal development may fairly be regarded as positively disproved in the insects and strongly discountenanced by the available evidence in the Crustacea. Apart from the *Stylops* difficulty, already sufficiently considered, this consideration appears to constitute the sole serious obstacle which has been found to the acceptance of the essentials of Smith's hypothesis, which otherwise fit the facts so well. It is necessary, therefore, to consider carefully whether it is really such a difficulty as it has been held to be.

In point of fact, it becomes clear on consideration that all single alteration can be found which can be safely interpreted as such a modification, though modifications of the male towards the female abound. If the parasites had any masculinizing tendencies at all we should expect, in view of the above considerations, that some unmistakable male characters would make their appearance in parasitized females. That they do not do so is an additional piece of evidence in support of the view that the parasites have no direct masculinizing effect whatever.

that the castration and transplantation experiments prove is that the external characters are differentiated independently of the gonad in normal development. Taken alone the results might appear to justify the deduction that the somatic cells are probably not alterable in any circumstances by any influence outside themselves. But the facts of parasitic castration alone show that such a deduction would be wrong. The sexual characters can be altered by an external influence, namely, parasitization, while the results of Kosminsky (1911 and 1924) and Emeljanoff (1924) in obtaining changes in the external characters of *Lepidoptera*, including inversions of the secondary sexual characters, by subjecting the pupae to abnormally high and low temperatures, and of Van Sommeren and others (Poulton, 1927) in producing gynandromorphs by subjecting pupae to a mechanical shock, show how other external factors may exert a more or less analogous effect. We are faced, then, by the fact that parasitization can cause a change of a kind which mere surgical castration and transplantation of gonads appears unable to produce. Presumably even the opponents of Smith's theory would admit that the change is effected by some sort of alteration of metabolism to or towards the female type. We may therefore consider whether it is possible to find any explanation as to why the parasite can induce such a change and the ovary graft cannot.

Now it is admitted that the somatic characters and the gonad are normally independent in their differentiation. Removal of the testis, therefore, does not influence the male somatic cells, which continue to metabolize in the male way. The ovary-graft is thus introduced into an environment which is definitively male. There may not be, and probably is not, anything positively inimical to the ovary in this, except that the male metabolism is not adapted to the manufacture on a large scale of the fatty and other material which the ovary requires for its full development. The ovary will therefore continue to develop, though possibly rather less vigorously¹ than in a female body

¹ In Meisenheimer's experiments the ovaries did not in fact develop very vigorously in the male body. He believed, no doubt rightly, that this was merely due to lack of space and not to any positive inhibition, but it might

on account of the shortage of nutrient material. This shortage will not be rectified because the male constitution of the somatic cells will be more than adequate to resist the demands made by the graft, which will not be strong enough, so to speak, to overrule their male predisposition. Similarly, we may suppose that gonad and soma in any one species are so adjusted to one another that in the abnormal individuals with female gonads and male soma the demands coming from the ovary are at no stage strong enough to overcome the constitutional—that is, presumably chromosomally determined—predisposition of the somatic cells to metabolize in the male way.¹ But supposing a stronger and more insatiable demand for the same nutrient materials was brought to bear on them: supposing at the same early age at which the grafts were introduced it were possible suddenly to establish a mature ovary in full and undiminished functional activity. We might well suppose that the male somatic cells, subjected to a much more insistent demand for these materials than they are adapted to withstand, might be forcibly swung over to the female type of metabolism in a way which the feeble little ovarian grafts are incapable of achieving.² Actually the establishment in the body of a young animal of an adult ovary in full activity is a manifest impossibility, but it is suggested that in nature an essentially similar experiment

also be in part a retardation due to deficiency of proper nutriment. Kopeć's results were somewhat similar (cf. foot-note No. 2 on this page).

¹ It is possible that after a certain stage the secondary sexual characters may become fixed and unalterable even if a change in metabolism does occur.

² It is perhaps necessary to state that there appears to be nothing in the results of the experiments on multiple transplantation of ovaries inconsistent with the views maintained above. In Kopeć's experiments (1911), for example, the ovarian tubes of female gonads established in the bodies of males were three to five times shorter than in normal females and the eggs were rather smaller with fewer and smaller yolk-granules. Several ovaries could be established in the body of one male, but the more there were the smaller they remained. This was attributed simply to the limitation of space in the male body, but the important point for present purposes is that there is no reason to suppose that the quantitative nutritional requirements of the normal ovary are ever significantly if at all exceeded in such cases.

is performed by substituting for an adult ovary a parasite whose demands are equivalent or at least substantially similar. In short, I submit that the solution of the whole difficulty is that the parasite brings to bear a drain of the same kind as, and not less drastic than, that caused by a fully adult ovary on the resources of an immature organism. If the matter is looked at in this fashion, there is seen to be no incompatibility between the gonad-grafting experiments and the view that the parasite reacts on the host like an adult ovary, which in all other respects seems so adequate; and I suggest that an interpretation of the phenomena of parasitic castration on the lines indicated provides in the present state of knowledge the most satisfactory and consistent hypothesis that can be put forward.

In conclusion, there are some minor considerations and possibilities of which brief note may be taken. We may observe, for example, the possibility that the parasite in withdrawing nutriment from the host may withdraw substances which are necessary to the maintenance of the male type of metabolism and which the organism is unable to replace sufficiently rapidly. In this way it might tend positively to inhibit the male type of metabolism at the same time as it favoured the female one. Though this is theoretically conceivable it is not, of course, at all a necessary assumption, and is merely noted for what it is worth.

If we imagine curves of production of male and female determining substances like those in Goldschmidt's well-known graphs we shall also recognize the probability that the relative concentrations of the two substances will differ much less in early life than in later stages, so that a change in metabolism from one sort to the other will be more easily effected in the young than later, as in fact there is every reason to believe is the case. Further, we may well suppose that in different forms the difference in the relative concentrations of these hypothetical substances in later life will be greater in some than in others, so that one form will be capable of being influenced up to a more advanced age than another. Following this line of reflection, it may be suggested that the singular local variability in

the extent to which crabs like *Carcinus* are influenced by *Sacculina* may be due to slightly different relative rates of production of the male and female determining substances in physiologically different races of the same crab, resulting in some reaching the stage at which modification is no longer possible sooner than others.

VII. SUMMARY.

1. The effects of Epicaridan Isopods on their hosts have not previously been studied in detail. *Gyge branchialis* at Naples is particularly suitable for this purpose on account of its commonness. In 1924 21.5 per cent. of *Upogebia littoralis* at Naples were found to be parasitized.

2. Some difference in the sex-ratio in old and young, parasitized and non-parasitized *Upogebia* are discussed. Amongst normal animals adult females outnumbered adult males by 3:2. Amongst young animals of about 3-6 mm. carapace length and parasitized material of all ages there was a marked excess of males. It is thought probable that there is in fact a real preponderance of males in the whole population, but that ovigerous females probably tend to remain nearer the mouths of their burrows and thus render themselves more liable to capture.

3. Fixation of the parasite normally takes place when the host is under 17 mm. long (carapace length: 6-6.5 mm.).

4. The available evidence suggests that the length of life of *Upogebia* is about (or at any rate not less than) three years. The life of the parasite is apparently normally coextensive with that of the host.

5. The effect of the parasite on the general vitality and viability of the host is negligible. Moulting and growth are not materially interfered with.

6. Parasitized males have chelae agreeing in size and appearance with those of the female and develop the appendages of the first abdominal segment, which are normally present in the female only. Parasitized females are unaltered externally, except that the chelae tend to average very slightly smaller

than in normal females. The genital pores are never obliterated in either sex.

7. The growth of the chelae in normal and parasitized animals, both absolutely and relatively to the body, are studied by means of graphs based on a large series of measurements. The parasitized male growth curves are closely approximated to the female type, while those of the female are not significantly altered. (For a fuller summary of the results on chela growth, see pp. 32-3.)

8. On account of the early age at which parasitization takes place all individuals are modified, in contrast to crabs parasitized by *Rhizocephala*, in which a considerable percentage of animals are unmodified, having evidently been parasitized too late in life.

9. Scattered oocytes occur amongst the male germ-cells in the testes of many normal males. These cells are never very numerous, but are seldom absent. They accompany the normal cells in their development and are eventually detached into the lumen of the testis, where they degenerate. They can sometimes be recognized amongst the older spermatogonia, but have not been traced with certainty farther back than this, so that it is uncertain whether they arise by metamorphosis of spermatogonia or are distinct from these from the outset. No evidence is found in *Upogebia littoralis* of any seasonal variability in the numbers of these cells.

10. Comparison is made with *Upogebia major*, which has paired abdominal extensions of the testis which appear to be constantly ovarian in character (Ishikawa), and *Upogebia stellata*, in which Runnström and the present writer have found similar posterior extensions of the testis containing oocytes at the hind end. *Upogebia stellata* may also develop rudimentary oviducts and female genital pores in the male.

11. The condition of the testis in parasitized males shows a wide range of variability from one in which it is only slightly reduced and spermatogenesis is proceeding vigorously to one of complete atrophy. The tendency to develop oocytes is much accentuated. They tend to become conspicuously more numerous and develop farther than in normal males, occasionally even

forming yolk. In a few cases a whole tract of testis appears to have been completely converted into ovary. Spermatogenesis may occur even in the oldest parasitized males and may continue locally even in greatly reduced testes; on the other hand, it may cease before reduction of the organ has proceeded very far. Sperm production and a marked development of oocytes may sometimes be found in different regions of the same testis. Rarely the lining of the vas deferens undergoes a more or less marked chitinization.

12. In the large majority of parasitized females the gonad is completely absent. When it persists in a reduced form it is always found to be an ovary in a state of diminished activity, but not otherwise abnormal.

13. The early age at which parasitization takes place results in the gonad pursuing its development from an early stage under the parasite's influence. The development of the testes in normal and parasitized males and females is outlined. Even normal animals show considerable variability in the degree of development of the testis at given body sizes. Males appear to be all sexually mature from about 6 mm. carapace length onwards, though they probably do not breed until later. Occasional cases of much earlier sexual maturity occur. Two males of carapace length 4 mm. were found to be producing ripe sperm. Females of 8-9 mm. carapace length may be found bearing eggs.

In parasitized males differentiation of the testis is markedly retarded and in some cases evidently completely checked, the ducts appearing to be in general rather less sensitive than the gonad. At a time not much antecedent to 8 mm. carapace length some of the less drastically affected individuals may begin to form sperm. In many, after a period of decreasing sperm production, spermatogenesis ceases entirely (cf. however, section 11).

14. The correlation between the condition of the gonad and the degree of modification of the secondary sexual characters and even between the different changes in the gonad itself is exceedingly loose.

15. Males with testes entirely absent may be the earliest

parasitized, but the variability in the modifications must be attributed largely to constitutional differences in the animals.

16. The modifications evidently persist, at least for a considerable time, if the parasite is removed ; but whether, in these circumstances, they are permanent is not established.

17. Two exceptional conditions are described :

- (1) An unparasitized male with female appendages. The condition of the gonad suggests that this is a case of natural recovery from parasitization.
- (2) Two (or three) parasitized males without female appendages. These might be supposed to be exceptional cases of very late parasitization, but this interpretation is impossible in one case and doubtful in the other. In the first the gonad is completely aborted ; in the second it is practically normal, but the chelae are markedly reduced. They are thought to be more probably constitutionally abnormal cases, where the organism has failed to respond to parasitization in the normal manner.

18. Previous work on parasitic castration is reviewed and discussed. Notwithstanding suggestions to the contrary, the modifications of sex characters in parasitically castrated Crustacea are exclusively in the direction of feminization, as is also the case in *Thelia* parasitized by *Aphelopus* (Kornhauser). The feminization is positive and unequivocal and cannot be interpreted as a return to or retention of primitive, undifferentiated, or juvenile features.

The solitary instance in which parasitization is accompanied by a definite exchange of characters between male and female is that of stylopization in Hymenoptera. It is concluded that the appearance in stylopized females of certain characters normally confined to the male must be due to some peculiar conditions controlling the development of these characters, and that their appearance is in some way dependent on the general nutritional disturbance and not on any directly masculinizing influence of the parasite.

19. Geoffrey Smith's theory that the parasite, by withdrawal of nutriment, reacts on the host in a similar manner to an adult

ovary is considered to be the only one which makes any approach to a real explanation. Objections to this theory on the ground of the experimental and other evidence against any effect of the ovary on the soma in Arthropods are considered. All that the castration and gonad-grafting experiments and the evidence from abnormal individuals with male soma and female gonads prove is that the somatic characters are uninfluenced by the gonad in normal development, and that gonad and soma are so adjusted to one another that in the above cases the demands coming from the ovary are at no stage strong enough to interfere with the constitutional (i. e. ? chromosomally determined) predisposition of the somatic cells to metabolize in the male way. But a more insistent drain on the same food materials might overrule this predisposition and compel an alteration of metabolism, and it is suggested that the parasite exerts its effect by bringing to bear a demand of the same kind as, and not less drastic than, that of an adult ovary on an immature organism.

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IX. EXPLANATION OF PLATES

All figures were drawn at table level with an Abbé camera lucida, Spencer 4 mm. objective and 6×ocular, except figs. 11 and 13, which were drawn with 16 mm. objective and 10×ocular. The original drawings are reduced by approximately one-third.

LIST OF ABBREVIATIONS

d.c., degenerating germ-cells; *d.cy.*, mass representing degenerated cytoplasm of a number of aborted oocytes; *d.e.*, degenerating eggs with masses of yolk granules; *f.n.*, follicle nuclei; *occ.*, oocytes; *oog.*, oogonia; *spc.*, spermatocytes; *spg. I*, primary spermatogonia; *spg. II*, secondary spermatogonia; *sptd.*, spermatids; *sptd. imm.*, young spermatids; *sptd. ad.*, advanced spermatids; *spz.*, spermatozoa; *v.d.n.*, vesicles representing degenerate nuclei; *y.*, yolk granules.

PLATE 1.

Figs. 1-4.—Typical sections of testes of normal male *Upogebia littoralis*, showing the main stages of spermatogenesis.

Fig. 1.—Section showing primary and secondary spermatogonia and nearly ripe spermatids; a few spermatozoa in the lumen.

Fig. 2.—Section showing (above) secondary spermatogonia and two oocytes, and (below) spermatocytes in synapsis and a small clump of primary spermatogonia; a few spermatozoa in the lumen.

Fig. 3.—Section showing primary spermatogonia, spermatocytes in a post-synaptic stage, maturation divisions, and spermatids. The main mass of the latter are in an early stage, others nearly ripe are seen nearest the lumen of the tube.

Fig. 4.—Section showing spermatocytes in a post-synaptic stage and an oocyte of the maximum size observed in an unparasitized male.

Figs. 5-8. Sections of testes of parasitized male *Upogebia littoralis*.

Fig. 5.—Section of a comparatively slightly affected testis, in which spermatogenesis is still in progress, but showing the tendency to loss of compactness in the arrangement of the cells characteristic of most parasitized testes (cf. fig. 2); slight local degeneration of cells.

Fig. 6.—Extensive degeneration: many spermatogonia not yet noticeably affected, later stages atrophied.

Fig. 7.—Complete degeneration: normal germ-cells entirely absent.

Fig. 8.—Section showing abnormal proliferation of follicle-cells without formation of germ-cells, sometimes observed in parasitized testes in an advanced stage of degeneration.

PLATE 2.

Figs. 9-11. Development of oocytes in parasitized testes of *Upogebia littoralis*.

Fig. 9.—Section showing production of oocytes on a much more extensive scale than ever found in unparasitized testes. The case selected for illustration is noteworthy because numerous spermatozoa are present in the lumen at the same time. A certain amount of spermatogenesis was still going on in other parts of the same gonad.

Fig. 10.—Section showing extensive development of yolk in oocytes in a parasitized testis. The larger egg-cell is beginning to degenerate.

Fig. 11.—Section showing conversion of a considerable tract of parasitized testis into ovary. Male elements appear to be entirely eliminated, though the gonad shows a much reduced state of activity as compared with a normal ovary.

Fig. 12.—Section showing cuticularization of the lining of the vas deferens, an occasional occurrence in parasitized testes.

Fig. 13.—Section of reduced ovary of a parasitized female, to illustrate the fact that in those cases where the female gonad does not atrophy completely its ovarian character is never altered: oogenesis proceeding up to a fairly advanced stage, larger eggs degenerating.

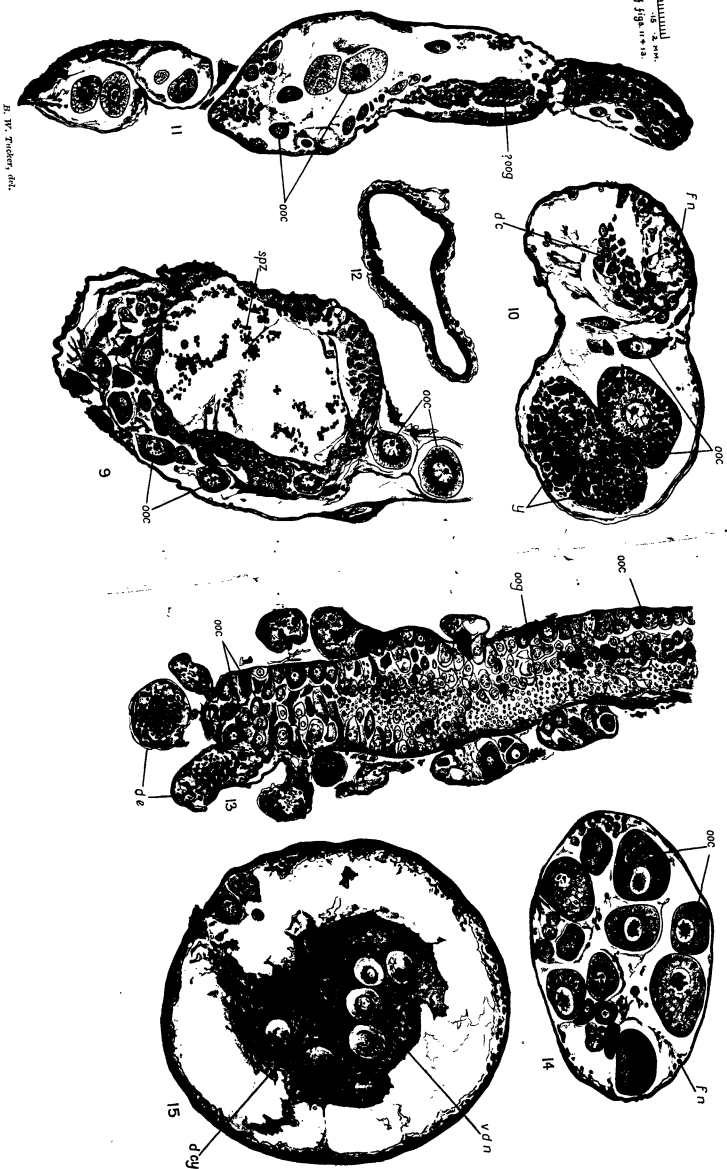
Figs. 14-15. Sections through the posterior ovarian region of the abdominal extension of the testis of *Upogebia stellata* collected in early March.

Fig. 14.—Section showing oocytes well developed, though displaying incipient degeneration.

Fig. 15.—Section showing an advanced stage in the degeneration of the oocytes. The cytoplasm of the eggs has broken down into a mass in which cell-boundaries are no longer distinguishable; the nuclei still persist as distinct vesicles.



lumen
Scale of figs. 11-13.



J. W. Truders, del.

On the unsegmented ovum of *Echidna* (*Tachyglossus*).

By

Professor T. Thomson Flynn, D.Sc.,

University of Tasmania.

With Plate 3.

INTRODUCTION.

THE account in the following pages deals with two eggs of *Echidna* (*Tachyglossus*) collected in Tasmania during the breeding season of 1929. They, with others, were acquired by the aid of a grant from the Grants Committee of the Royal Society, to which committee and to Professor J. P. Hill, who aided me in obtaining this grant, I express my appreciative thanks.

As is well known, material of the developmental stages of *Echidna* is not easy to get, and the matter is being made more complicated by the increasing scarcity of the animal. I have altogether some thirty intra-uterine stages, but even so the series can hardly be said to be complete. It is hoped to fill the existing gaps in the series, so far as possible, by further collecting during the season of 1930.

The known forms of *Echidna*¹ are regarded as belonging to one species, *Echidna aculeata* Shaw, of which there are three well-authenticated varieties, with a possible fourth. These are as follows: var. *typica* or *aculeata*, found on the mainland of Australia; var. *lawesi*, inhabiting Southern New Guinea; and var. *setosa*, confined to Tasmania. The fourth variety, *multiaculeata*, described by Rothschild in 1905, from the southern portion of South Australia, is of doubtful validity.

¹ I have used the widely known generic name *Echidna* in preference to the more correct term *Tachyglossus*.

Up to the present, as might be expected, practically all work dealing with the development and organogeny of *Echidna*, contained in the writings of Owen, Caldwell, Semon, Hill, Gatenby, and others, has been based on material of the variety *typica*.

My own material, being obtained wholly in Tasmania, is of the variety *setosa*, of which but one developmental stage has been recorded. This was a pouch egg, which, with the contained embryo, is now preserved in the collection of the Tasmanian Museum. It was briefly described by Morton in the 'Papers and Proceedings' of the Royal Society of Tasmania for 1887 (1888).

No detailed description of the unsegmented ovum of *Echidna* has yet appeared. Apparently Semon (1894) collected some material of this period of development, but it has not yet been investigated. Caldwell (1887, p. 472) states that an unsegmented ovum which he took from the lower end of the Fallopian tube measured 3.2 mm. in diameter, and of this egg he gives a description of the membranes. In his Pl. 31, fig. 1, he also gives a drawing of a vertical section through the germinal disc, but there is no description in the text, and it is not possible to make out from the figure any details of the structure of the disc or of the stage of development at which it has arrived.

As yet, the only full account published of the structure of the unsegmented monotreme ovum is that of Gatenby and Hill (1924), dealing with an intra-uterine egg of *Ornithorhynchus*. Previously to this, Gatenby had referred somewhat briefly to the same egg (1922). Gatenby and Hill, in addition to giving a full description of the arrangement of the white and yellow yolk and of the structure of the latebra, deal in detail with the germinal disc and with the distribution and significance of certain nuclear structures which they found in close association therewith. Since it will be necessary to refer to the work of these authors from time to time in the course of the present paper, it is advisable to do no more at this juncture than draw attention to the fact that they came to the important conclusion that the ovum of *Ornithorhynchus*, like that of *Sauropsida*, is polyspermatic, and further, they state

that the two polar bodies in *Ornithorhynchus* are of unequal size, the first being larger than the second, and undergoing division before the latter is completely separated off.

As will be seen from the results of the investigation of the two stages described in the present paper, *Echidna* agrees with *Ornithorhynchus* in that the first polar body is larger than the second, but it remains undivided. As for polyspermy, it is apparently not a normal occurrence in *Echidna*.

The egg of *Ornithorhynchus* examined by Gatenby and Hill was cut in sections in toto; but, with my own material, owing to the difficulties associated with the brittleness and general impermeability of the yolk, I felt that such a technique involved too much risk to the germinal disc. The two eggs here described were first fixed in Bouin's picro-formol-acetic mixture, after which, by the judicious use of needles and a small brush, all but the immediately underlying yolk was removed from the germinal disc. The latter, after being double-embedded in celloidin and wax, was cut into sections, each of 5 micra. The sections were stained in Heidenhain's iron-haematoxylin.

Ovulation in *Echidna*.

Owen (1881) describes and figures the left uterus of an *Echidna* containing three eggs, and Broom (1895), in his note on the period of gestation of this animal, records a total of two eggs as having been laid on successive days. Nevertheless, in the many specimens of the Tasmanian variety which I have had, there never has been more than one egg present at one time. Semon had a similar experience with the mainland variety, so that it would appear that any other condition is abnormal.

It has also been stated that the right uterus never becomes pregnant. Thus, in speaking of the group generally, Semon (1894, p. 62) says: 'Obwohl bei beiden *Monotremengattungen* sich sowohl im rechten als auch im linken Ovarium Eier entwickeln und ausbilden, werden doch nur die Eier des linken Ovariums vollkommen reif und gelangen nach Austritt aus dem Ovarium, wo die Befruchtung stattfindet, in den linken Ovi-

duct.' This statement, which has been adopted by Weber (1928, Bd. ii, p. 39), does not apply to the Tasmanian variety of *Echidna*. In this form, so far as my results go, about one-third of the developing eggs are to be found in the right uterus. It is possible that the case is different with the variety found on the mainland of Australia, but it must be remembered that, even here, Owen (1881, p. 1052) records an egg from the uterus of the right side, and Hill and Gatenby, only recently (1926, p. 746), have described a perfectly normal corpus luteum as being present in the right ovary of an *Echidna* which had just previously laid an egg.

Measurements of Eggs.

Gatenby and Hill (1924) have made some remarks on the variation in size of eggs of *Ornithorhynchus* and *Echidna*, particularly in the early intra-uterine stages, and have come to the conclusion that such variations are due partly to differences in the size of the ovum, partly to variations in the thickness of the albumen layer.

But it appears that few, if any, of these measurements have been made on living eggs. Such measurements are fairly constant, but they vary tremendously after fixation whatever be the fixative used. As will be seen hereafter, several types of fixing solution have been employed in this work, but the effect in all is practically the same. The shell becomes wrinkled at first, and later becomes separated from the yolk-mass by a space of variable extent, artificially caused by the absorption of fluid from the exterior. The albumen layer becomes greatly swollen and disorganized. That this is the case is shown by the following series of measurements taken before and after fixation. The eggs are all in early segmentation stages and the measurements of the living eggs are, I believe, the first to be placed on definite record.

I would look upon the egg of *Ornithorhynchus* of which a photograph is shown in Plate I, fig. 1, of Wilson and Hills's paper (1907), as a typical example of the kind. Here, the yolk-mass is seen to be separated from the outer shell membrane by a space which is more or less obliterated on one side, evi-

dently that on which the egg was resting during fixation and preservation.

<i>Egg.</i>	<i>Total diameter.</i>		<i>Fixative.</i>
	<i>Fresh.</i>	<i>After fixation.</i>	
	mm.	mm.	
i	4.5	6.0	Bles.
ii	4.0	5.5	Formol-bichromate-acetic.
iii	4.0	5.6	Formol-bichromate-acetic.
iv	4.0	5.6	Dubosc-Brasil.
v	4.4	5.3	Bles.

Under these circumstances, it is evident that measurements of the total diameters of eggs, taken after fixation, must be regarded with a large amount of caution.

DESCRIPTION OF MATERIAL.

Egg No. 1.

The diameter of this egg, measured intact and in the living condition, was 4 mm. The shell, before removal, was seen to be extremely delicate and transparent, its thickness in sections being 0.0018 mm. The albumen resisted the swelling usually associated with fixation and varied somewhat in thickness, being as much as 0.03 mm. over the germinal disc, while it is as little as 0.017 mm. over parts of the remainder of the egg.

The removal of these two layers exposes the yolk-mass with the germinal disc on one side, enclosed in the very delicate zona. The diameter of the ovum deprived of its membranes is, therefore, as near as can be judged, 3.95 mm. This measurement is much in excess of that given by Caldwell (3 mm.) as the diameter of the oviducal ovum of *Echidna* (see also Hartman, 1929, Tables 6 and 8).

Below the zona, in the sections, is to be seen a somewhat vacuolated layer of varying consistency which appears to be a coagulum, possibly produced as a result of fixation.

It is usually stated that the yolk of the monotreme egg is yellowish in colour and that the blastodisc is therefore easily distinguishable from the remainder of the ovum. I have not

found this to be the case with the Tasmanian variety of *Echidna*. Here, when fresh, the yolk is perfectly white, and it is practically impossible to make out, through the shell, the position of the germinal disc. With fixing fluids containing picric acid, such as Bouin's solution, the yolk stains yellowish, and so the blastodisc stands out a little more prominently. But the use of most other types of fixatives makes it necessary to stain the egg in toto before the position of the disc can be determined.

The structure of the germinal disc in this egg and its relationship to the yolk, latebra, &c., are shown in fig. 1, Pl. 3. Externally is to be seen the shell (*sh.*), at this stage very thin and delicate, below which is the laminated layer of albumen (*alb.*).

Underlying this again is the zona pellucida (*z.p.*).

The arrangement of the yolk is similar to that found in the unsegmented egg of *Ornithorhynchus* (Hill and Gatenby, 1924) with some differences in detail. As in *Ornithorhynchus*, the 'yellow' yolk is disposed in two zones, an outer and an inner, while there is present over the whole surface, except at the germinal disc, an extremely delicate layer of very fine yolk-spheres. The germinal disc (*g.d.*), more flattened and of greater extent than is the case in *Ornithorhynchus*, overlies a much vacuolated portion of the ovum, the nucleus of Pander (*nu.P.*), composed of finely granular material which does not stain with haematoxylin and which is very similar to the material of the disc itself. The nucleus of Pander is continuous below with the latebra (*lat.*), which is like that of *Ornithorhynchus* as described by Hill and Gatenby. Surrounding the nucleus of Pander is a ring-like mass of fine grained 'white' yolk. Into the upper side of this mass projects a circular thickening of the lower side of the germinal disc to be referred to later.

The germinal disc differs greatly in appearance and shape from that described for *Ornithorhynchus*. Compared with that of the latter genus, the disc of *Echidna* is much more definite in outline, more flattened from above down, and more expanded laterally.

Viewed from the surface in the unsectioned ovum, the disc

is quite circular and presents a central transparent area surrounded by a darker ring. Its diameter, measured over all after being embedded and cut into sections, is 0.55 mm., more than ten times the surface diameter in the only comparable stage of *Ornithorhynchus*. Great as this discrepancy is, it is perhaps not surprising if we take into account the very remarkable changes which may take place in the disc of meroblastic eggs during maturation processes. These have been described in some detail by Harper for the pigeon (1904, see particularly figs. 4a, 5, and 8, Pl. 1).

In *Echidna*, the upper side of the disc is gently convex. On its lower side the disc, although unmistakably defined, is markedly irregular in its contour. It thins out at its outer edge and is of no great thickness (0.016 mm.) in the centre, but, about half-way between the two, there is an annular zone where the disc projects downwards into the yolk, its thickness here being as much as 0.043 mm.

These differences in the thickness of the disc account for the appearance of its division into a lighter area surrounding a darker one.

The disc is composed of a very fine, granular, homogeneous material from which yolk is practically entirely excluded, except along the lower edge and the margins, where the disc is to some extent invaded by yolk-spheres. The substance of the disc is entirely similar in structure and staining qualities to the material which constitutes the basis of the much vacuolated nucleus of Pander.

Nuclear Phenomena.—The ovum is in the last stage of maturation, the second polar body having just been given off. The two polar bodies are situated near the centre of the disc and are much smaller than in *Ornithorhynchus*. They are also very unequal, the second being quite minute. The first polar body measures 0.022 mm. \times 0.012 mm. and is found to occupy three consecutive sections each of 5 micra; the second measures 0.0075 \times 0.0055 mm. and is found in two sections only. Fig. 3, Pl. 3, is a drawing, much enlarged, of the two polar bodies and the female germ nucleus.

The female germ nucleus is a flattened mass of deeply

staining chromatic material, the chromosomes being clumped. It measures 0.005×0.0027 mm. and is almost in contact with the surface of the ovum which, at this point, has a slight out-bulging. It would appear as if the separation of the second polar body had but just been completed. In the latter body, the chromatic material is arranged somewhat more openly, but it is impossible to make out details.

I am unable to find any trace of the sperm nucleus in the disc. The series of sections is quite complete, so that there is a possibility that the ovum has not been fertilized. This is very unlikely since numbers of spermatozoa are to be found in the albumen layer. It is rather more likely, as occasionally happens at this stage, that the male sperm nucleus is indistinguishable from the general mass of the disc.

Nor are there to be found in the disc any accessory sperm nuclei, marginal or otherwise. Examination of this and the next succeeding stage has convinced me that polyspermy does not normally occur in the fertilization processes of *Echidna*.

Egg No. 2.

This egg was obtained by one of my students (Mr. F. D. Cruickshank, B.Sc.), who was good enough to take charge of the removal of eggs from animals during my temporary absence from Hobart. The egg was not measured fresh, but in its swollen condition after fixation had a diameter of 4.6 mm. Its diameter after removal of the envelopes was 4 mm.

Sections of the disc show that development is at a stage not before described for any monotreme, that of conjugation of the germ nuclei.

Structure of the Egg.—The shell membrane (fig. 2, *sh.*, Pl. 3) is thin, being on the average about 0.003 mm. in thickness. Thin as it is, it has gained much in strength and in consistency since the last stage. It is much more definitely contoured and, being firmer, is more brittle, sometimes causing considerable trouble in the process of sectioning. Owing to alterations brought about during fixation, the thickness of the albumen layer (*alb.*) varies greatly, but in places is reduced to 0.0075 mm.

The germinal disc.—Viewed superficially the disc is no longer circular but is elliptical, with a long diameter of 0.49 mm. and a short one of 0.37 mm. It therefore occupies a surface area less than that of the disc of the stage previously described. This is to be attributed, no doubt, partly to individual variation, partly to alterations in the distribution of the substance of the disc itself. The loss of surface area is associated with an increase in the depth of the disc, which now measures in thickness in the centre 0.05 mm.

The disc (fig. 2, *g.d.*, Pl. 3), in section, is definitely biconvex in shape, the centre being its deepest portion. Its upper surface fits against the zona (*z.p.*) while the lower one is unevenly edged, passing over somewhat abruptly into the much vacuolated nucleus of Pandér (*nu.P.*) below. The somewhat profound alterations which have taken place in the shape and size of the disc call to mind once again the changes which take place in the germinal disc of the pigeon's ovum as described by Harper (1904).

The substance of the disc is finely granular and is quite homogeneous except at the margins, where there is some slight penetration by yolk granules. At one end of the disc, however, that to which the pronuclei are nearer, the upper portion of the disc contains a well-marked and distinctive layer of yellow yolk-spheres, or, in other words, the end of the disc is here buried beneath a layer of such spheres. Such a phenomenon occurs in the pigeon, as can be seen by referring to Harper's paper (1904, figs. 12 and 13). As will be seen later, the difference in the composition of the two ends of the disc, together with the position of the germ nuclei and of the polar bodies, gives a definite and unmistakable bilateral symmetry to the undivided germinal disc.

In sectioning, an endeavour was made to keep parallel to the long axis, with the result that the conjugating pronuclei and the polar bodies are in the same sections. Altogether there are 75 sections in the series. The pronuclei are to be found mainly in sections 36 to 38, so that their position is quite median, but they are situated nearer one end of the disc than the other. They are placed one-third the distance from that end of

the disc which is superficially invaded by the yellow yolk-spheres.

There is, therefore, a definite bilateral symmetry and, in shape and size, the disc approximates closely to that of *Ornithorhynchus* at an early stage of segmentation. Such a stage has been described and drawn by Wilson and Hill (1907, Text-figs. 1 and 2). Their drawings show an elliptical disc divided into eight cells arranged symmetrically, four on each of a median axis which obviously corresponds to the long axis of the unsegmented disc of *Echidna*. The eight-celled stage of *Ornithorhynchus* would appear to measure approximately 0.6×0.5 mm. It is, therefore, comparable in size with the disc of *Echidna*.

From these considerations, supported by conclusions to be drawn from the situation of the pronuclei, it is reasonable to suppose that the first segmentation division in the ovum of *Echidna* takes place along the longitudinal axis, dividing the disc into two equal areas. Unless, however, there is a decided rearrangement of the nuclear material of the disc, it is obvious that the succeeding division must be unequal. This point will have to be settled on a subsequent occasion by reference to early segmentation stages.

The Pronuclei.—These are in the stage of conjugation and of partial fusion. Each is in the shape of a flattened bean-shaped body, the two being closely attached along the flattened sides where there is a tendency towards disorganization along the line of union. This line of attachment is tangential to the surface of the disc, so that one of the nuclei is nearer to the surface than the other. The more superficial is larger and paler. It measures 0.01×0.021 mm. The second pronucleus, situated more deeply, measures 0.007×0.019 mm.

The pronuclei are similar in appearance to those of the pigeon as depicted by Harper in his fig. 12, but the stage of *Echidna* here considered is somewhat more advanced. Of the two germ nuclei (fig. 5, Pl. 3), the larger is invested by a definite membrane which, however, shows signs of breaking down at each end. In the case of the smaller germ nucleus, the nuclear membrane has largely lost its contour and has

become wrinkled and shrunken. The region of the ooplasm immediately surrounding this nucleus is loosened, contains irregular spaces, stains rather lightly, and in general presents evidence of a distinct loss of nuclear fluid through the disorganized membrane.

There are appearances under the high power which suggest the emission, from both nuclei, of chromidia in the way described for the mouse and the rat (Kremer, 1924, figs. 12 and 18, 21 and 22), but of this one cannot be certain.

In the large pronucleus the chromatin takes the form of a loose network beset with rather coarse granules. The chromatin of the small pronucleus has the same arrangement but, owing to the loss of nuclear fluid which has taken place, the whole nucleus stains darkly.

Polar Bodies.—These are two in number, the first body remaining undivided. They are situated at the centre of one end of the disc, the end farthest from the pronuclei. As in the last stage they are of unequal sizes, the larger measuring 0.017×0.0093 mm. and occupying three consecutive sections of 5 micra each while the smaller measures 0.0095×0.0075 mm. and is found in two consecutive sections. Each is somewhat flattened and consists of material of similar composition to the plasm of the disc, with an ill-defined nucleus.

There is no trace, in any part of the disc, of accessory sperm nuclei or of the results of their division.

SUMMARY OF CONCLUSIONS.

1. In this communication is described for the first time the unsegmented germinal disc of the monotreme *Echidna* (*Tachyglossus*), and measurements are also given of living intra-uterine eggs at early stages.

2. Segmentation is not initiated in *Echidna* until the egg has arrived in the uterus.

3. The polar bodies are much smaller than those described for *Ornithorhynchus*. The first is much larger than the second but, contrary to what has been reported to occur in *Ornithorhynchus*, remains undivided.

4. The germ nuclei, immediately before the formation of the first cleavage spindle, undergo conjugation and fusion.

5. Contrary to what is described for *Ornithorhynchus*, polyspermy does not normally occur in the fertilization of the ovum of *Echidna*.

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EXPLANATION OF PLATE 3.

LETTERING.

alb., albumen; *g.d.*, germinal disc; *g.n.♀*, female germ nucleus; *lat.*, latebra; *nu.P.*, nucleus of Pander; *pb 1*, first polar body; *pb 2*, second polar body; *sh.*, shell; *w.y.*, white yolk; *y.y.*, yellow yolk; *z.p.*, zona pellucida.

Fig. 1.—A vertical median section through the germinal disc and adjoining parts in Egg No. 1. The second polar body is to be seen at the

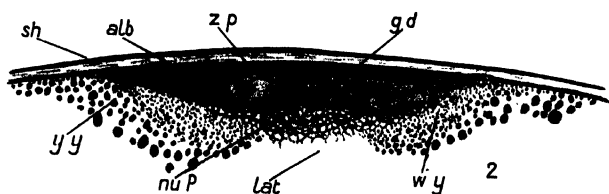
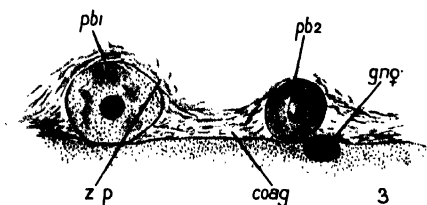
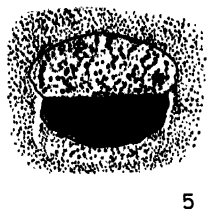
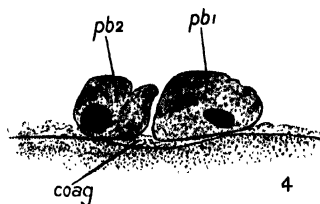
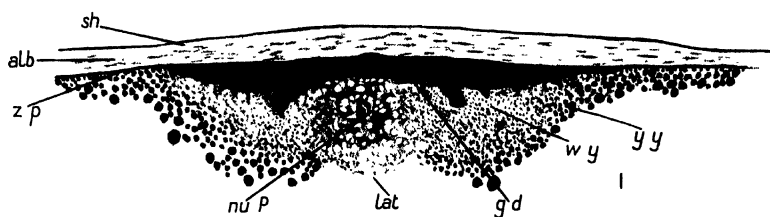
centre of the surface of the disc while the female germ-nucleus is included in the disc just below and to the right of the polar body. $\times 40$ (approx.).

Fig. 2.—A similar section through Egg No. 2. At the extreme margin of the disc (at the right side in the figure) can be seen the minute polar bodies while, within the disc towards the other end are the conjugating germ nuclei. $\times 40$ (approx.).

Fig. 3.—A drawing composed from five consecutive sections showing the female germ-nucleus and the polar bodies of Egg No. 1. In this and the next figure *coag.*, coagulated material between the zona pellucida and the surface of the ovum. $\times 400$ (approx.).

Fig. 4.—The two polar bodies of Egg No. 2. $\times 400$ (approx.).

Fig. 5.—The conjugating germ-nuclei of Egg No. 2. $\times 400$ (approx.).



Development of the Sex Glands of Calotes.

I. Cytology and Growth of the Gonads prior to Hatching.

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With Plates 4, 5, 6.

THERE are two dissimilar and opposing views regarding the origin of the sexual elements in the vertebrates in general, and these dissimilar views are held by workers upon the origin of the reproductive elements in the Reptilia as well. The provisional conjectures are these: first, the forerunners of the propagative elements, the so-called primordial germ-cells, originate outside the fundament of the gonad and migrate to that incipient structure throughout the course of early ontogenesis; second, the definitive germ-cells arise in the gonad, concomitant with its formation from units directly derived from the germinal epithelium.

The first view was clearly set forth in the pioneer work of Nussbaum (1880), and the second was probably first enunciated by Waldeyer (1870). Both men worked on animals other than the reptiles. It was not until Allen (1906) traced the migration of the germ-cells in *Chrysemys* that work was specifically done upon the Reptilia. Allen maintained that the primordial germ-cells of the turtle arose in the hypoblast and migrated through the entoderm of the gut, and then through the mesoderm of the dorsal mesentery to the incipient gonad. He considered these primordial germ-cells to be extra-regional forerunners of the definitive sexual elements and were essentially

alike during the indifferent stage of embryonic development, but they suffered different fates in the course of sexual differentiation. In the male the migratory cell became incorporated into the sex-cords and developed into sperms, while in the female the early sex-cords degenerated along with the primordial elements that were incorporated into them; those primordial germ-cells, however, that did not become involved in the degenerating sex-cords developed into ova. According to Allen the primordial germ-cells give rise to both somatic and germinal stuff.

Dustin (1910) called the extra-regional forerunners of the definitive germ-cells gonocytes, and explained that they degenerated after migrating to the gonad. Other cells, he believed, arose from the germinal epithelium which developed into the procreative elements. He divided the generations of germ-cells into two, primary and secondary. The primary generation of germ-cells, which are probably the same things as the primordial germ-cells, arose before the anlage of the gonad was even formed, in a region that was not clearly defined. These primary cells remained in the medial edges of the lateral mesodermal plates until the anlage of the gonads was formed. Immediately the primary germ-cells began to migrate into the fundament and degenerate. The secondary generation of germ-cells arose from the germinal epithelium, bearing no relation whatever to the first, and differentiated into the definitive ova and sperms.

Von Berenberg-Gossler (1914) found, in the lizard (*Lacerta agilis*), only the secondary generation of gonocytes, those that arose from the germinal epithelium. He ventured the assertion that the *Urgeschlechtszellen*, the so-called primordial germ-cells, or the cells of Dustin's primary generation, were not germ-cells at all, but were wandering entoderm cells moving out of the entoderm to swell the ranks of the mesenchyme cells.

Jordan (1917), from the loggerhead turtle, adduced evidence that supported a part of Allen's original contention, especially that part dealing with the indifferent stage. Jordan believed that the primordial germ-cells, extra-regional forerunners of the propagative cells, originated early in some unknown region of

the embryo and migrated over a route similar to that of *Chrysemys* to the gonads, where they directly transformed into either ova or sperms. This is the most extreme contention that has been reported, for there was recognized neither a primary nor secondary generation of gonocytes, and no difference was recognized in the fate of the primordial cells in the two sexes.

Simkins (1925), in *Trionyx*, was unable to find evidence supporting the assumption that the cells of the germ-line have an extra-regional origin. It is quite doubtful whether there is such a cell as the primordial germ-cell at all; in the sense that such cells are the forerunners of the definitive sexual elements that arise in regions remote from the gonad, and migrate thereto over a longer or shorter period of time. It was found in *Trionyx* that the definitive sexual cells arose from the germinal epithelium.

There is no general agreement, among advocates of the view that the primordial germ-cells are segregated early, either upon the time of their origin or as to just where the cells arise. There is likewise no agreement concerning their fate. The preponderance of opinion now supports the contention of Waldeyer that the reproductive elements arise from the germinal epithelium, by a process of differentiation of somatic cells into germ-cells. The opinion is growing that both the large and small units in the early gonad share in the process of differentiation. In the female sex the large cells often go into the formation of ova while the small cells in the male transform into spermatogonia.

The origin of the definitive procreative elements involves the cytological differentiation of the gonad and does not necessarily involve the primordial germ-cell, since such a cell, if present at all, degenerates in most instances and hence cannot be taken as a forerunner of the sexual elements. The postulation of such a cell, or collection of cells, becomes as unnecessary as the degeneration, since the actual units used in building the reproductive gland are derived in the last analysis from that portion of the peritoneal epithelium immediately covering the genital thickening. Granting that such is the case, the problem of the embryonic differentiation of the gonads is in many cases unsolved. The present paper deals with the origin of the gonad

and its cytological differentiation from the first day of laying to hatching.

The material was collected and preserved by the junior author.

MATERIALS AND METHODS.

The materials used in the present study consist of a collection of embryos of the Agamid lizard, *Calotes*, collected by the junior author in the vicinity of Ahmedabad. The eggs were incubated in the laboratory, and at the desired time the embryos were removed and fixed in a variety of fixatives, of which the modified Bouin gave the best results.

The incubation period was found to be slightly in excess of six weeks, so for convenience of treatment the collection was arranged into six groups, representing intervals of one week. In the early stages the interval was found to be too large, so the early embryos are regarded as being so many days old, i.e. following the time of laying. Such accurate timing was rendered possible by frequently surprising the female on the nest and recovering the eggs at once. At other times gravid females were captured in the field and the eggs removed by slitting the oviduct.

OBSERVATIONS.

The eggs of *Calotes* undergo considerable development before they are laid. Embryos obtained from eggs immediately after laying, and from eggs removed from the oviduct as well, vary from 2 to 2.5 mm. in crown rump length. In these early embryos the fundamentals of most of the organs are well advanced. The neural tube is closed except the anterior and posterior neuropore, the optic vesicle is formed, and the fore- and hind-gut folded off. The mesonephric body extends along the dorsal roof of the body-cavity from the posterior region of the lung-buds to the caudal limit of the coelom. Along the ventromedian border of this mesonephric body there is a thickened layer of cells which extends from the cephalic to the caudal end. The anterior portion of this thickening may be considered the anlage of the cortex of the suprarenal gland and the posterior,

the anlage of the sexual gland. It is impossible, at this early age, to tell where one leaves off and the other begins. Transversally this thickening extends from the root of the mesentery to the crest of the mesonephric swelling. The epithelium of the mesonephros, other than over the thickened area, is composed of a single layer of cells, which merges laterally into the epithelium of the body-wall and medially into the radix mesentericus. Everywhere the nuclei of the cells composing the epithelium and the thickened layer beneath are ovoid or spherical, of small size, deeply staining, and crowded closely together.

The cells of the thickened area are of the same general types, but indicate a more active mitosis by the increased frequency of division figures. This increase in the rate of proliferation is responsible for the thickening, which in some places bulges medially far enough to impinge against the dorsal mesentery. Associated with the small cells of the dorsal body-wall are to be found large masses of yolk incorporated into more or less definite limits. In such units an irregular structure resembling a nucleus can be discerned, whose size, however, is no greater than that of surrounding nuclei. These large masses (fig. 11, Pl. 5) are to be found anywhere from the cephalic end of the thickening to the caudal longitudinally, and from the mesentery to lateral body-wall transversally. They are as numerous in the cortical portion of the swelling as they are in the genital, and so cannot be considered special types or even forerunners of the sexual elements. Compared in size to the smaller units they are enormous, and contain more or less closely aggregated granules of material that stains intensely red in eosin and intensely black in osmic acid (fig. 16, Pl. 6, and fig. 1, Pl. 4). In this manner they resemble cells of the wall of the yolk sac. Their peripheral limits are not always distinctly demarcated and rarely regular, although the contour is usually rounded, even in cases where the form is plainly pyramidal. The large granules, which are probably modified yolk spherules, occur always in that part of the cell occupied by the cytoplasm whose ground-work stains lavender with haematoxylin, as does the structure identified as the nucleus. Of particular interest are these tentative nuclei; they are rarely regular in outline, always pale and

dimly seen, and in many places do not seem to have a nuclear membrane at all.

The red (or black, depending on whether eosin or osmic is used) spherules have been no doubt derived from the yolk and represent modified vitelline materials which have been incorporated into them by direct contact with the yolk-mass. Because these spherules have been conceived as yolk and are included in elements resembling cells, they have been called nurse-cells, with the reservation, however, that they may not conform to a rigid definition of a cell.

The nurse-cells differ so markedly from the others among which they lie that they can be identified quite easily, even under low-power magnification. These nurse-cells have been found in embryos of the first day in the nephrocoel, in the lumen of the fore- and hind-gut (never in the epithelial walls), in the epithelium of the mesonephros, and in the swelling that may either give rise to the cortex of the suprarenal or the genital anlage. There may be an important significance in the fact that the nurse-cells found in the lumen of the gut are much smaller than those found elsewhere, and they stain a more brilliant red in eosin. It is singular, too, that the nurse-cells occur more numerous in those regions of the embryo which are in open communication with the mass of yolk in the yolk-sac.

The entire thickening on the ventromedian border of the mesonephros is shown in fig. 1, Pl. 4. The smaller and larger units entering into its structure are shown, and is typical of any section cut through the mesonephros from the caudal to the cephalic end. One is unable to differentiate the suprarenal from the genital anlage.

On the second day of incubation the ventromedian swelling is somewhat thicker and the nurse-cells occur in bunches, and are rarely met with outside the thickened area. The smaller units are more numerous and the mitotic figures occur more often in the periphery of the thickening.

On the third day the nurse-cells have all disappeared from regions outside the ventromedian thickening, and many found within the thickening show unmistakable signs of retrogression. They are smaller, the granules are not so large, stain feebly and

diffusely. The smaller cells of the thickening have multiplied until the layer is now several cells in thickness, probably showing a slightly thicker posterior than anterior end.

In embryos of the fourth day the nurse-cells are confined to the thickening, and for the next three days very little change takes place in the shape of cytological constituents of the cortical and genital anlagen. The nurse-cells continue to dissipate until their size is very little greater than the somatic elements derived from the epithelium. As the nurse-cells become progressively smaller and smaller and poorer and poorer in yolk materials, they take more and more of the haematoxylin and less of the eosin.

During the first week of incubation the epithelial thickening consists of a single part having more than one layer of cells. The nurse-cells are most frequently found in the peripheral part of this layer, which during all this time undergo no divisions but progressively dissipate. The number of smaller cells is augmented by the division of cells mainly derived from the germinal epithelium, and this division progresses outward, centrifugally, not inward or centripetally. Up to this time, too, the longitudinal mass presents no differentiation into a strict cortical and genital area, but this condition is soon changed.

The anterior part of the ventromedian thickening does not keep pace with the posterior part and, also, the cephalic portion increases so as to involve more and more of the root of the mesentery and does not extend so far laterally on the mesonephric body. This cephalic part has been taken to be the cortex of the suprarenal gland, while the thicker more caudal part must be the anlage of the gonad. One part merges imperceptibly into the other, but they begin to acquire characteristic differences which will be brought out later on.

The sexual part of the thickening, the gonogenic mass, now tends to become differentiated into medullary and cortical layers (fig. 11, Pl. 5). At the close of the first week there is little difference between the cortex and the medulla of the gonad: the chief distinction is that the medulla is more compact and appears of slightly greater density than the cortex. The

cells are of equal size, though one finds the nurse-cells only in the cortical layer (fig. 11, Pl. 4).

During the second week of incubation the nurse-cells diminish in size and number and are confined strictly to the cortex of the gonad and of the suprarenal. The yolk-spherules, which were at first large globular masses, fragment, and in embryos of eleven days appear as fine granules surrounding an indefinitely delimited nucleus (fig. 12, Pl. 6). Later, as the yolk-granules become more finely divided, the mass takes on a cloud-like effect and is diffused into the cytoplasm. By the end of the second week the nurse-cells have become so reduced that one meets them only occasionally. One still finds them in the lumen of the gut, in the walls of the yolk-sac, and in the primordium of the liver.

The close of the second week witnesses another important development. A new type of cell begins to make its appearance and stands out so distinctly that some space will be devoted to its description. This new cell arises in the cortex and is clearly demarcated from the surrounding cells. Its cytoplasmic membrane is absent, but the nucleus is much larger than other cells and the cytoplasm immediately around it is pale and lightly staining (figs. 5, 7, 15, Pls. 4 and 6). This cell is undoubtedly a primitive germ-cell and first appears during the second week of development. Such cells have been found in the cortex of embryos of the eleventh day (fig. 15, Pl. 6), and from that time on to hatching their role in the formation of the gonad can be traced successfully.

These primary germ-cells are not to be confused with the so-called primordial germ-cell. They have been designated here as primary in the sense that they may not transform directly into the definitive eggs and sperms, and also their behaviour in the two sexes may not be the same. They arise from enlarged cortical cells and are confined, for the most part, to the posterior part of the cortical thickening, although one cannot tell for sure whether any may be in that part of the thickening which forms the cortex of the suprarenal gland. By the end of the second and well into the first part of the third week the cortex of the gonad can be definitely told from the cortex of the

suprarenal by the presence of these germ-cells; for they are then strictly confined to the cortex or germinal epithelium (fig. 7, Pl. 4). It is impossible to recognize, in embryos earlier than the eleventh day, a pregonocytic progenitor of these primary germ-cells. No cells that compare with them in general or particular appearance exist anywhere in the embryo prior to their origin on the eleventh day. Some of them may form earlier than we have discovered, and in a few instances we have noticed indications of them as early as the ninth day, but always in the cortex and never in regions remote from the fundament of the sex gland and suprarenal cortex.

The only conspicuous elements along the dorsal body-wall before the primary germ-cells arise are the nurse-cells, and we shall show later on that these units bear only an indirect relation to the germ-cells. The origin of the primary germ-cells is conceived to be a gradual one; they do not spring suddenly into being but enlarge slowly until they assume the proportions sufficient to render them conspicuous. Somatic cells derived from the germinal epithelium proliferate to form the cortex, and these cortical cells enlarge throughout the entire period of incubation to form the primary germ-cells.

At the end of the third week the nurse-cells have all disappeared from the genital swelling, and the fundament enlarges by multiplication of cells in the medulla and cortex. The medulla is the thicker (fig. 7, Pl. 4). The rate of growth shifts during the third week and the greater activity takes place in the cortex towards its close: the shift in growth soon results in a fundament whose medullary and cortical parts are of equal thickness. There are no nurse-cells to be found in the fundament and the number of gonocytes has greatly increased, which causes the anlage to bulge into the body-cavity.

The disappearance of the nurse-cells can be followed with precision; as a general rule the units decrease in size. The granules of yolk that were at first quite large begin to fragment and apparently to dissolve until they appear as diffuse clouds about a more or less indistinct nucleus. Finally, the clouds are no longer detectable and the nurse-cell ceases to be identified in any part of the embryonic body, except perhaps in the

fundament of the liver. The stages in the dissolution of the nurse-cells can be better understood and graphically visualized if the figs. 16, 20, 17, 12, and 13, Pl. 6, are compared in the order given.

During the fourth week the rate of growth is greater in the cortex than in the medulla, and it takes on cytological characters that differentiate it completely from the core of the fundament. The cells of the cortex are large (fig. 8, Pl. 4), dispersed, and surrounded by very clear areas and cytoplasmic mantles that are denser away from rather than proximal to the nucleus. The medulla, on the other hand, is composed of much smaller cells, closely packed, darkly staining, and containing only an occasional large cell. From the periphery of the cortex certain cells are seen to lie detached and directed towards the body-cavity as if they had been pushed out by the enlarging fundament. This condition is constantly met with in the development of the gonad of *Calotes*, and it is so striking that it suggests the possible direction of growth of the entire fundament. In a former paper (Simkins, 1925), the senior author mentioned certain down-growths of cells from the germinal epithelium of *Trionyx* in a centripetal manner that gave rise to the primary germ-cells. If there is a movement of cells in the opposite direction, then the fundament increases by a centrifugal and not a centripetal growth, and the correct expression would be an outgrowth rather than an ingrowth.

The identity of the germinal epithelium, that is, its identity as a distinct and additional layer of the cortex, is lost. Indeed, the entire cortex seems to be a thickened germinal epithelium which reposes as a sort of cap over the medulla. The mesenchyme cells lying between the germinal epithelium and the tubules and glomeruli of the mesonephros proliferate to form the core of the distending cortex. Certain cells from the inner surface of the cortex become involved in the medulla and help increase its bulk. From the fourth week onwards the distinction between cortex and medulla is so well marked, and the preponderating types of cells are so markedly different, that a very extensive interchange of cells between the two portions of the sexual primordium seems quite unlikely. Both portions pursue independent rates of growth by multiplication of their cells.

In embryos of the fifth week the genital anlage increases in size, the lateral and medial folds become more marked, and the attachment to the mesonephros is reduced to a relatively narrow mesovarium (mesorchium). The cortex does not change greatly, it is somewhat thicker in some cases and in others does not seem to have increased much. The medulla exhibits a tendency to diverge along two lines of differentiation. In one type the cells are arranged in irregular rows from the hilus outward (fig. 10, Pl. 5). These rows end in masses of cells against the space that separates the medulla from the cortex. In the second type (fig. 2, Pl. 4) certain cells of the medulla arrange themselves into incipient rods, or solid cords, not at all unlike the beginning stages in the formation of the seminiferous tubules as observed in other forms (Simkins, 1928, 1928). In the earlier stages (fig. 8, Pl. 4) this cord-like arrangement was not discernible, hence the solid massed arrangement of the medullary cells is taken as the more undifferentiated condition and the cord-like arrangement as the tendency towards higher differentiation. But there are embryos, also of the fifth week, in which no cord-like arrangement of the medullary cells can be seen. The explanation of this seeming anachronism is possibly due to a delay in differentiation, presaging the formation of the female sex.

In general, the cortex shows very little change in structure; in those cases, however, where the medulla tends to form the incipient tubules it is not so thick as in cases where no such tubules or cords are present. Its cells rapidly divide as shown by the frequency with which mitotic figures are met, and in those cases where no tubules are found in the medulla there is formed a layer of very small cells around the periphery of the cortex (fig. 10, Pl. 5), which are in striking contrast to the germ-cells. This is taken to be an epithelial layer and may be germinal, although the activity seems to be in the cells below it.

During the sixth week the embryos hatch, and at that time the gonads are quite large and hang from the dorsal body-wall by the mesentery, but the gonads at hatching are not all of the same size and all do not present the same cytological picture in cross-section. In one type the gonad is smaller, the medulla relatively thick with many incipient tubular or cord-like structures. The

other type has fewer cords and the cortex is very thick and the gland as a whole is larger (fig. 9, Pl. 5). One is struck by the absence of a well-defined germinal epithelium around the cortex. Indeed, the whole cortex (fig. 9, Pl. 5) appears to be a greatly hypertrophied germinal epithelium. It is quite sharply marked off from the medulla and is composed, for the most part, of large cells actively engaged in dividing. The smaller cells are inconspicuous in both number and size.

Sexual differentiation is not completed at hatching, but there is a definite tendency for the gonads to lean decidedly towards the male or female sex. This tendency is revealed in the two types of gonads that can be recognized at hatching, one type with a relatively thin cortex and a thick medulla whose small cells surround certain larger cells in the form of incipient cords, and a second type with thick cortex and relatively thin medulla (fig. 9, Pl. 5) in which the sex cords are reduced to a minimum or are not present at all. The former type probably results in the formation of a male and the second a female.

In a bird's-eye view of the development of the gonads of *Calotes* one notices that the anlage of the cortex of the suprarenal gland and of the genital thickening are intimately related, so intimately in fact that several days of incubation must pass before one can be sure which is which, and in which there are large conspicuous nurse-cells that dissipate during the third week and seem to take no part in the formation of the cortex of either the suprarenal or the gonads.

Along with the disappearance of the nurse-cells the primary germ-cells arise from the somatic cells which were products of the germinal epithelium. As the gonads grow in size, which seems to be accomplished by cells pushing out centrifugally, they differentiate into two portions, a medullary and cortical, both of which may play an important role in the process of sexual differentiation later. It is suggested that the further development of the cortex at the expense of or with the diminution of the medulla forms a female, while the augmented medulla at the expense of the cortex results in a male.

DISCUSSION.

The presence of yolk in the epithelium of the body-cavity during the very early stages of development may have some significance other than indicating that the tissues including such yolk once enjoyed a close contact with the yolk-sac and yolk-mass. If it is permissible to look upon the genesis of the gonad as an expression of the rate of growth, the nurse-cells might conceivably be looked upon as serving to supply the necessary fuel for such increased metabolism. The fact that the nurse-cells were found to remain longer in the genital thickening might favour such a view, and yet the disappearance of the nurse-cells from the cortex of the suprarenal precedes that of the gonads so shortly that increased metabolism must be extended so as to include the formation of both cortices. There might have been an accidental incorporation of the nurse-cells into the epithelium of the body-cavity that has no particular significance at all.

It is quite definitely shown that the nurse-cells have no relation whatever with the primordial germ-cell. There is no evidence that the nurse-cells ever move about. They are impassively caught and remain in that place where they are entrapped until their substance dissolves and the nucleus fragments. In a former study the senior writer (Simkins, 1925) pointed out that the retention of yolk by cells for a longer or shorter time was not a reliable characteristic of germ-cells. And, also, the presence of large nurse-cells so widely scattered, in such remote parts of the embryo, leads one to believe that they were passively incorporated there and destined to become resorbed *in situ* without ever moving anywhere else. There is no evidence whatever that the nurse-cells are migratory.

The germ-cells that arise in the cortex come from no more remote regions than the germinal epithelium. If the figs. 15, 19, 14, and 18, Pl. 6, are studied in the order mentioned the origin and growth of the germ-cell can be appreciated at once, and if such upward growth is compared with figs. 16, 20, 17, 12, and 13, Pl. 6, which show the dissolution of the nurse-cell, the differences in the two processes and their independency can be correctly seen.

Whether or not the large cells of the early gonad, designated

the primary germ-cell, ever metamorphoses into definite ova and sperm has not yet been demonstrated. It is possible that they do not, because in many forms the smaller units of the gonads (Hargitt, 1925, 1926) have been shown to be the direct forebears of the definitive reproductive cells.

The significance of the relation of the medulla to the cortex may be of consequence, in that it would serve as a basis for the explanation of sex intergrades embryologically, in which case a sex intergrade could be looked upon as consisting of an equal proportion of cortical and medullary portions more or less intimately intermingled. The fate of the two portions, cortex and medulla, will be dealt with in detail in the forthcoming study on the development of the sex-gland from hatching to maturity.

The general hypothesis that definitive ova and sperms have extra-regional forerunners in far-off places of the embryo is not supported by the experimental evidence now accumulating. Janda, 1929, has reported unmistakable evidence that in the segmented worm, *Uridrilus*, both male and female germ-cells regenerate from somatic cells. Stohler, 1928, finds that there is seasonal regeneration of the germ-cells from the peritoneal epithelium in the European toad, and the junior author has determined a like seasonal regeneration of the germ-cells in *Calotes*. The evidence from castrations is conflicting. Hanson and Heys, 1929, report some cases of regeneration of the ovaries in the rat. The conflict seems to be one of definition of the word and concept of regeneration. If regeneration is defined as the restoration of the original form after mutilation, then there can be no doubt that in the mammals there is a high percentage of regeneration, but if regeneration of ovaries is defined as the formation or transformation of differentiated peritoneal epithelium into an ovary, then no such thing occurs.

The excellent work of Humphrey, 1928, by no means clinches the solution of the problem in favour of the primordial germ-cell. In his series of experiments on the embryos of *Amblystoma*, the mesoderm containing the early gonads was removed along with the whole lateral window in the abdominal wall. After the lapse of a certain length of time he could find no evidence that germ-glands reformed on the operated side. If the embryos had been

reared to sexual maturity and found sterile or lacking one gonad on the operated side, greater weight could be attached to the conclusions. On the other hand, there is no assurance that the germinal epithelium, which is the sole source of the germ-cells, was left intact. Of course, if the germinal epithelium is removed, the somatic source of the germ-cells is gone, and there could be no sex-cells or sex-gland formed.

The evidence now building up concerning the phenomena of sex reversal does not support the belief that sex is irrevocably fixed, or that the germ-cells are set apart early in ontogeny. Such a view denies the influence of the hormones and the rate of metabolism on the determination of sex and the formation of the sexual elements.

The forerunners of the definitive sexual cells arise from the germinal epithelium after the anlage of the gonad is formed, and during the course of development some become modified into eggs, some degenerate, and in some cases the smaller cells of the reproductive gland directly change over into the procreative elements.

SUMMARY.

1. The ventromedian thickening on the mesonephric body gives rise to the cortex of the genital gland and the suprarenal.
2. Nurse-cells are to be found in this thickening, which disappear during the third week of incubation, without influence on the germ-cells.
3. The primary germ-cells arise in the anlage of the gonad while the nurse-cells are degenerating, from elements derived from the germinal epithelium.
4. The gonad at the fourth week is composed of a medullary and cortical portion, the former composed of small cells, some of which begin to arrange themselves into incipient cords before hatching; the latter is composed of large cells and probably represents the thickened germinative epithelium.
5. There is a tendency towards sexual differentiation at hatching, but sex is not yet definitely established.

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EXPLANATION OF PLATES 4-6.

ABBREVIATIONS.

art.ren., renal artery; *cl.g.*, germ-cell; *cl.mt.*, mitotic cell; *cl.so.*, somatic cell; *cl.vt.*, nurse-cell; *cr.*, cortex of the gonad; *e'th.g.*, germinal epithelium; *e'th.pi'th.*, peritoneal epithelium; *m.*, medulla of the gonad; *sph.vt.*, yolk spherule; *tbl.*, incipient tubule.

PLATE 4.

Fig. 1.—Cross-section of the genital ridge, first day after laying. $\times 1000$.

Fig. 2.—The gonad, 40 days of incubation. $\times 750$.

Fig. 3.—Cortex of the gonad during the fourth week of incubation. $\times 750$.

Fig. 4.—Germ-cells in the cortex at hatching. $\times 1500$.

Fig. 5.—Germ-cells from the cortex, 16 days' incubation. $\times 1500$.

Fig. 6.—Enlarged cell from the medulla, 16 days' incubation. $\times 1500$.

Fig. 7.—Cortex of the gonad at 16 days' incubation. The medulla is not shown but would occupy the space in which fig. 5 is placed. $\times 750$.

Fig. 8.—A portion of the cortex and medulla of the gonad at 30 days' incubation. Free cells moving centrifugally. $\times 750$.

PLATE 5.

Fig. 9.—Cross-section of the gonad at hatching. $\times 750$. The enlarged germ-cell at end of leader *cl.g.* $\times 1500$.

Fig. 10.—Cortex and medulla of a gonad at 30 days' incubation. $\times 750$.

Fig. 11.—Cortex of the gonad at 7 days' incubation. The stroma and that part destined to form the medulla, not shown. $\times 1000$.

PLATE 6.

All figures drawn to the scale of 1500 diameters magnification.

Fig. 12.—Nurse-cell at 12 days' incubation.

Fig. 13.—Nurse-cell at 20 days' incubation.

Fig. 14.—Germ-cell at 28 days.

Fig. 15.—Germ-cell at 11 days.

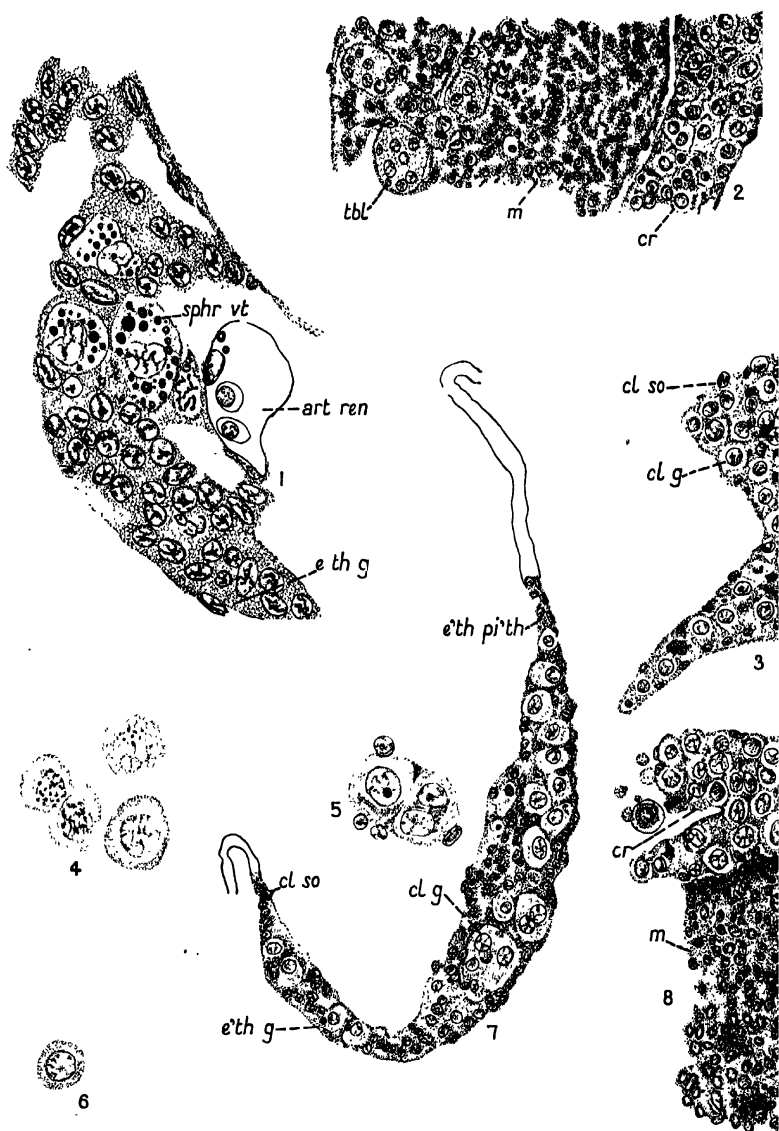
Fig. 16.—Nurse-cell on the first day after laying.

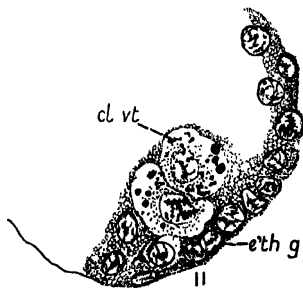
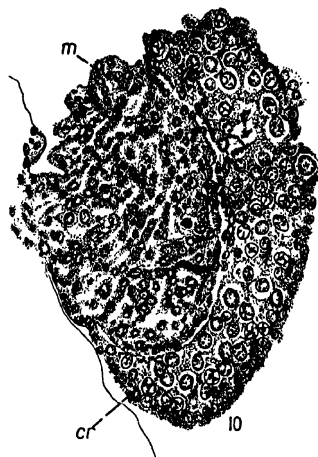
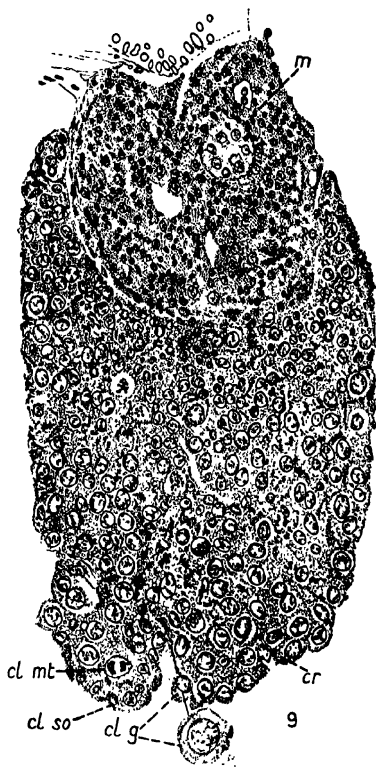
Fig. 17.—Nurse-cell on the eleventh day.

Fig. 18.—Germ-cell at hatching.

Fig. 19.—Germ-cell at the sixteenth day.

Fig. 20.—Nurse-cell on the seventh day.







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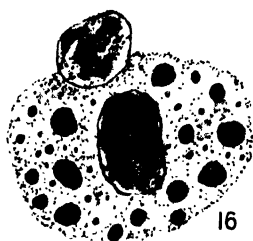
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20

A modified Gold Chloride method for the demonstration of Nerve Endings.

By

Fred W. Cairns,

Institute of Physiology, University of Glasgow.

With Plates 7 and 8.

DURING a series of histological investigations into the nerve-supply of certain skeletal muscles, which have from time to time been undertaken in this laboratory, great difficulty has been found in obtaining constant and satisfactory results.

The experience of this laboratory has been that, on the whole, the gold chloride methods give the greatest promise of reliability, and an attempt has been made to determine, so far as possible, the conditions necessary for a technique which will give uniformly good results. Many modifications of the existing techniques were tried with little success.

Eventually, it was found that the method described below yielded results both constant and satisfactory.

MATERIAL REQUIRED.

Fresh lemon juice (filtered), 1 per cent. gold chloride (British), formic acid 1.22 s.g., pure glycerine, distilled water, bone or wax-tipped forceps, clean cloth, and some small bottles.

All solutions must be made up in distilled water, and only small quantities of the various solutions (except glycerine) should be used. Excess fluid is positively harmful; simply cover the tissues.

Strict cleanliness is essential; rinse all bottles with distilled water before using and between each change.

METHOD.

1. Excise small pieces of muscle, e. g. cat's intercostal, place in a solution of 1 part formic acid and 3 parts filtered lemon juice and leave in the dark for 10 minutes.

2. Decant solution, place tissues within the folds of the clean towel, and press gently to absorb all excess fluid.

3. Transfer tissues to 1 per cent. gold chloride and return to the dark for another 10 minutes. At the end of this period, remove excess fluid by the method described in step 2.

4. Transfer tissues to 25 per cent. formic acid and keep in absolute darkness for 24 hours.

5. Again repeat operation 2 and pass to pure glycerine. Darkness is now no longer necessary.

The tissues may be kept in glycerine for months, if desired, without any sign of deterioration and probably with advantage.

EXAMINATION OF TISSUES.

After the tissues have been for some hours in glycerine, place a portion on a microscopic slide, immerse in glycerine, cover with a cover-glass, and examine with the $\frac{3}{4}$ objective. The muscle-tissue will be found to be graded into certain differently stained areas, usually a clear part on the outside, then a part coloured bluish violet to red, and finally, if a large piece of muscle has been taken, a more or less opaque central part. The most productive parts are found where the clear area merges into the blue. Make repeated change of focus and field until a part is found showing nerve-fibres or end-plates. The nerve-elements, when found, are stained an intense black colour.

Note the part where there is an abundance of these stained fibres. Remove the cover-glass carefully, then gently separate off, from the remainder, the part selected. Remount in glycerine. If necessary, on examination, repeat this process of selection.

It will be noted that teasing the tissue is not advised, as this may break up the large tree-like processes of the nerve-fibres.

The specimen thus obtained may be rendered permanent by ringing the cover-glass with a mixture of equal parts warmed Canada balsam and melted paraffin wax or some other suitable ringing medium.

Although this method is chiefly intended for the demonstration of motor nerve-endings in skeletal muscle, it is, as the photomicrographs (which have not in any way been retouched) show, very effective with other types of nerve-fibres and endings.

Incidentally, it may be noted that if one desires to demonstrate the myenteric plexus, either the muscle-coats of the intestine should be separated off before treating the tissues, or alternatively remove as much of the mucous membrane as possible before treatment and strip off the muscle-coats when in glycerine. This can easily be managed with a pair of forceps. The intestine of the rabbit should be used in preference to that of the cat, as the intestinal muscular coat of the latter is too thick.

My thanks are due to Professor E. P. Cathcart and to Dr. A. McL. Watson for their kindly advice and help. I wish also to thank Mr. J. R. Bell for the patience and care he has taken in preparing the photomicrographs.

March, 1930.

DESCRIPTION OF PLATES 7 AND 8.

- Fig. 1.—Node of Ranvier (cat). $\times 250$.
- Fig. 2.—Motor end-plates : intercostal muscle (cat). $\times 60$.
- Fig. 3.—Motor end-plates : intercostal muscle (cat). $\times 250$.
- Fig. 4.—Nerve-endings : gluteus maximus (cat). $\times 250$.
- Fig. 5.—Part of muscle spindle : intercostal muscle (cat). $\times 250$.
- Fig. 6.—Organ of Golgi : gluteus maximus (cat). $\times 60$.
- Fig. 7.—Organ of Golgi : gluteus maximus (cat). $\times 250$.
- Fig. 8.—Pacinian body : mesentery (cat). $\times 60$.
- Fig. 9.—Auerbach's plexus : small intestine (rabbit). $\times 60$.
- Fig. 10.—Auerbach's plexus : small intestine (rabbit). $\times 250$.
- Fig. 11.—Meissner's plexus : small intestine (rabbit). $\times 250$.
- Fig. 12.—Ganglion cells : urinary bladder (cat). $\times 60$.



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12

The development of the Club-shaped Gland in *Amphioxus*.

By

Edwin S. Goodrich, F.R.S.,

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of Oxford.**

With 10 Text-figures.

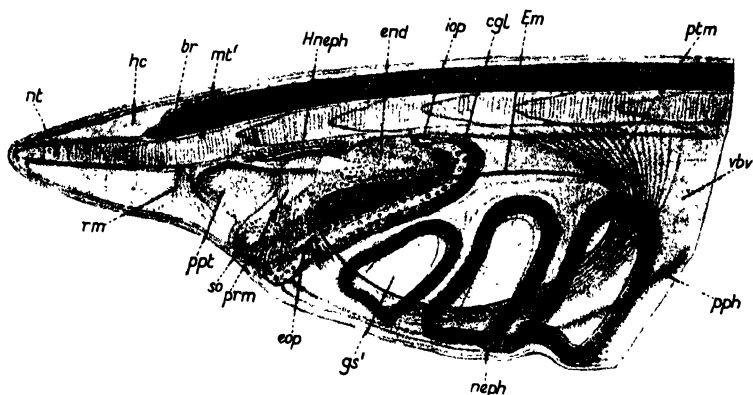
THE club-shaped gland of the larva of *Amphioxus* has attracted much attention since its development was first studied by Kowalevsky in 1877 (4), who believed it to be derived from the first mesodermal segment. Hatschek, in his classical work published in 1881 (3), corrected this error, and described the origin of the gland from a transverse thickening of the endodermal wall of the gut on the right side opposite the mouth in the region of the first myotome. He states that, in the embryo of about 10 segments, this thickening extends dorsally up the right side, and ventrally on to the left side; that it becomes folded, a groove being formed on its inner surface; and that by the closing of the lips of the groove it finally becomes separated off as an elongated sac from the gut ('Gegen das Ende der embryonalen Periode erfolgt der Verschluss der Rinne und die Abschnürung dieser Bildung vom Darne', p. 74). This sac on the right of the gut has a narrower prolongation to the left, which soon acquires an opening to the exterior just below the edge of the mouth (see Text-fig. 1). In the larva, then, the gland, whose wall is now formed of a layer of characteristic large cubical granular cells, opens by means of a duct partly ciliated and lined with flattened cells; while at an earlier stage it is supposed to be a closed sac ('der ganzen Länge nach zur Abschnürung gekommen', p. 75).

Lankester and Willey, 1890 (5), describe the gland in the larva with only three 'primary' gill-slits (definitive left series) as possessing an external but as yet no internal opening. The

latter opening into the gut becomes established later, according to them, and is present high up on the right side in the larva with twelve 'primary' slits. This condition persists until, as Willey showed (9), the whole gland disappears at metamorphosis.

In 1897, R. Legros (6) again studied the development of the club-shaped gland. According to him it arises from an endo-

TEXT-FIG. 1.



Enlarged left-side of view of larva with eleven open 'primary' gill-slits. *br*, brain; *cgl*, club-shaped gland; *em*, dorsal edge of mouth; *end*, endostyle; *eop*, external opening of gland; *gs'*, first 'primary' gill-slit; *hc*, head cavity; *Hneph*, Hatschek's nephridium; *iop*, internal opening of gland; *mt'*, first myotome; *neph*, nephridium; *nt*, notochord; *pph*, peripharyngeal ciliated tract; *ppt*, preoral pit; *prm*, preoral muscle; *ptm*, postoral muscle; *rm*, rostral muscle; *so*, preoral sense-organ; *vbv*, ventral blood-vessel.

dermal thickening on the right which becomes hollowed out, the cavity so formed acquiring an internal opening into the gut already in the larva with one gill-slit. The gland, he says, 'naît comme une masse endodermique pleine qui se creuse ensuite d'une cavité et s'ouvre secondairement dans le tube digestif', p. 528. Legros further throws doubt on the existence at any stage of an external opening, and maintains that the

appearance of a pore figured by Willey is probably due to a defect in the section.

The correctness of this surmise was strongly denied later by MacBride, 1898 (7), who emphatically and rightly stated that an external opening exists in the larva.

Gibson, 1910 (2), 'never found any trace of an external opening' in his larvae, and stated that his youngest larva 'does not yet possess an internal opening'.

J. W. van Wijhe, 1914 (8), confirmed Legros's discovery of an internal opening in the young larva with one slit, and MacBride's statement that an external opening is also present. He correctly described a narrow zone of flagellated cells between the glandular region and the duct in the fully developed organ.

As for the morphological significance of the club-shaped gland, it has been variously interpreted. Willey (10) maintained that it represents the antimere of the first 'primary' gill-slit (of the definitive left series); its internal opening on the right side being taken as evidence of this homology. Van Wijhe (8), however, considered that the mouth on the left and the gland on the right represented the first pair of gill-slits; though he appears to have accepted Legros's account of the secondary development of the lumen and its inner opening. Van Wijhe further held that this first pair of slits lies between the mandibular and hyoid segments like the spiracular slits of Craniates.

Recently, Garstang, 1929 (1), has compared the gland to the budding stolon of Ascidians; but he appears to have been under the erroneous impression that 'the bulk of the gland is derived from the left, not the right, side, and that the right internal orifice is obviously not the original orifice of evagination', p. 140.

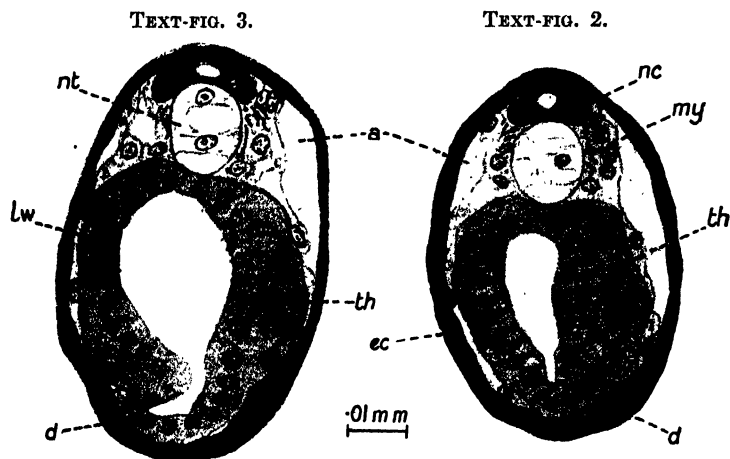
Of the function of the club-shaped gland little appears to be known beyond the fact that its secretion passes out by the external pore and is apparently carried into the mouth. Unfortunately I have not been able to make observations on the living larvae.

From the brief summary given above it will be seen that there has been considerable difference of opinion as to the exact mode of origin of the club-shaped gland and of its openings,

and it is the object of this paper to give the results of a reinvestigation of the development undertaken in the endeavour to reconcile and correct previous accounts.

For the material used I have to thank the authorities of the Stazione Zoologica of Naples, who provided me some years ago with embryos and early larvae fixed in corrosive acetic and in Flemming's fluid. Later stages I obtained myself from the Pantano at Messina in 1902.

The rudiment of the club-shaped gland first appears as a thickening of the right endodermal wall of the gut in embryos

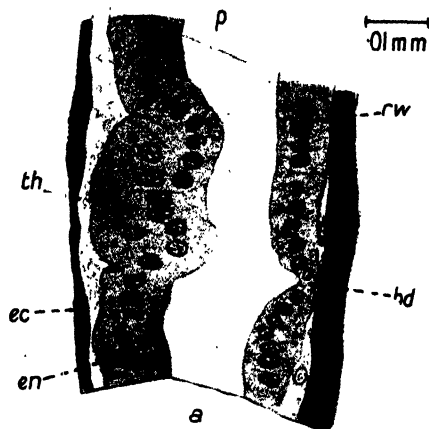


2. Transverse section of an embryo at an early stage when the mouth is not yet pierced and the buccal diverticulum is scarcely marked.
3. Transverse section of a slightly older embryo. *a*, artifact space; *d*, ventral diverticulum, rudiment of duct; *ec*, ectoderm; *lw*, left endodermal wall of gut; *my*, myotome; *nc*, nerve-cord; *nt*, notochord; *th*, endodermal thickening on right wall of gut, rudiment of glandular region of club-shaped gland.

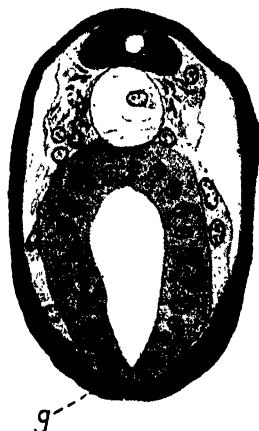
without any distinct sign of mouth. By the time the position of the mouth is indicated by a small evagination of the left wall of the gut, the slightly more posterior transverse thickening on the opposite side is well defined just posteriorly to the rudiment of the endostyle (Text-figs. 2, 3, 5).

It is important to notice that neither at this stage nor in later stages does the thickened rudiment of the gland pass ventrally beyond the mid-ventral line; never, so far as I have observed, does it extend on to the left wall of the gut. At the stage when the buccal endodermal diverticulum is growing outwards, but the mouth has not yet been pierced, the thick-

TEXT-FIG. 5.



TEXT-FIG. 4.

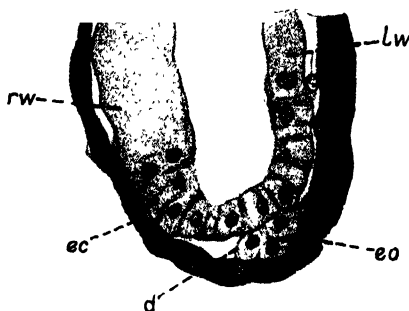


4. Transverse section of same embryo as shown in Text-fig. 3, cutting through the developing first 'primary' gill-pouch, *g*.
5. Longitudinal frontal section of an embryo at about the same stage. *a*, anterior, and *p*, posterior. *bd*, endodermal buccal diverticulum on left; *ec*, ectoderm; *en*, rudiment of endostyle; *th*, thickening of endoderm of right wall of gut, rudiment of glandular region of club-shaped gland.

ening bulges slightly into the lumen of the gut and still more to the outside. Below it, in the mid-ventral line, there develops a diverticulum of the endoderm into which extends a prolongation of the lumen of the gut (Text-figs. 2, 3); this is the first rudiment of the duct to the exterior. It will be noticed that this outgrowth closely resembles the rudiment of a 'primary' gill-slit, as shown, for instance, in the case of the first 'primary' slit developing more posteriorly (Text-fig. 4). Almost from the first the distal end of the rudiment of the duct bends and grows

round to the left and forwards. By the time the mouth and first gill-slit have become pierced, the tip of the rudiment has fused on the left with the ectoderm preparatory to the formation of an opening to the exterior below the mouth (Text-fig. 6). Meanwhile, the transverse patch of modified epithelium has become thicker, and its inner surface has become grooved. The cells composing it have multiplied, become pressed together, and

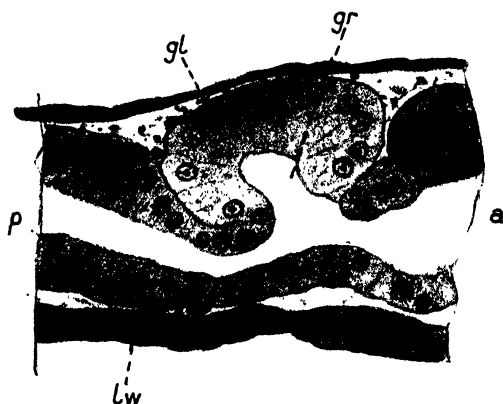
TEXT-FIG. 6.



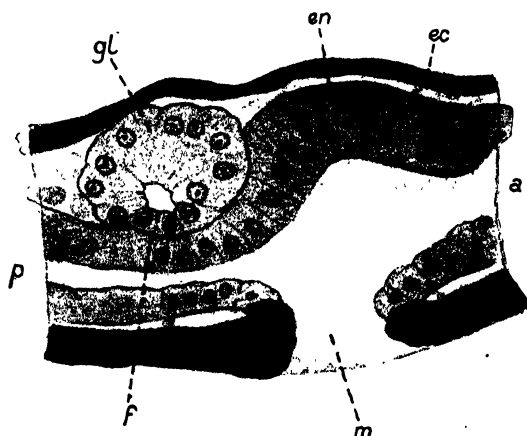
Ventral portion of a transverse section of an embryo at the stage when the mouth and first gill-slit have just been pierced. *d*, tip of ventral diverticulum fused with ectoderm, *ec*; *eo*, external opening just forming; *lw* and *rw*, left and right walls of gut. The section passes immediately behind the mouth.

in section appear set irregularly in several rows (Text-fig. 5). There is, however, no hollowing out of a lumen. As the groove deepens the cells again spread out in a single layer, enlarge, and become granular (Text-fig. 7). The anterior and posterior edges of the groove become more prominent and folded towards each other, so that they finally meet to form a tubular gland enclosing a lumen (Text-figs. 7, 8). This folding off of the tube must, of course, take place so as to preserve its communication with the developing duct. Comparison of transverse with longitudinal sections shows that the closing off of the gland occurs chiefly from below upwards, ventro-dorsally (Text-figs. 7, 8, 9). The two edges of the groove meet in a ventral fold which passes to the left of the mid-ventral diverticulum, and this fold grows

TEXT-FIG. 7.



TEXT-FIG. 8.

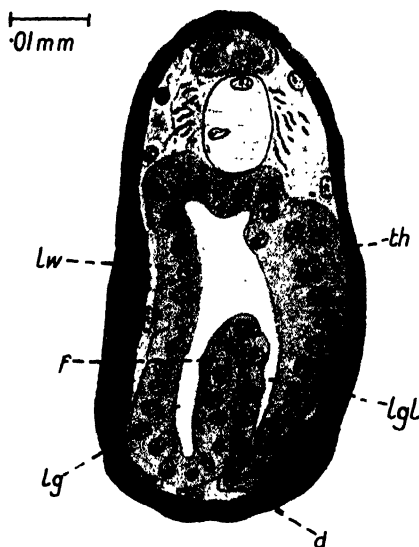


Longitudinal frontal sections of a later embryo with round open mouth, *m*. *a*, anterior, and *p*, posterior; *f*, fold rising from below; *gl*, glandular tube; *gr*, groove on inner surface of endodermal rudiment. The section shown in Text-fig. 7 is more dorsal than that shown in Text-fig. 8. Magnification as in Text-fig. 9.

upwards, separating the lumen of the now tubular gland from that of the gut. Dorsally the dividing fold is never completed, but leaves permanently open the communication between the two lumina. The manner in which the gland is folded off is

shown in the three diagrams appended (Text-fig. 10 A, B, C). Thus the lumen of the gland is derived from the original cavity of the gut, and the internal opening of the gland is the persistent communication between them. It remains open, as I have been able to observe, throughout larval life, and does not close until the gland itself ceases to function and disappears at metamorphosis.

TEXT-FIG. 9.



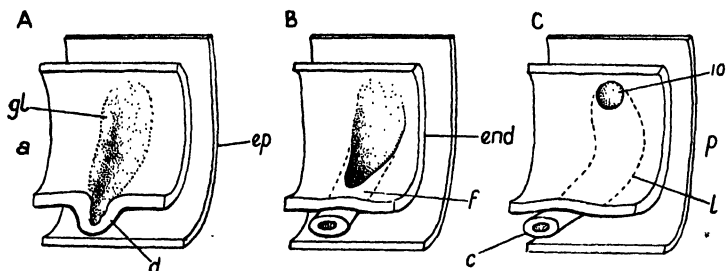
Transverse section behind the mouth through an embryo at the same stage as that shown in Text-fig. 7. *d*, duct to exterior which is continued forwards; *f*, uprising fold separating lumen of gut, *lg*, from lumen of glandular region, *lgl*; *lw*, left wall of gut; *th*, endodermal thickening, rudiment of glandular region.

In the fully developed tubular club-shaped gland three regions can be distinguished: (1) on the right, the glandular tube opening dorsally from the gut; (2) ventrally, the short flagellated region passing towards the left to (3) the duct leading to the external pore just below the anterior edge of the mouth.

Concerning the general morphology of the club-shaped gland I have little to say beyond this, that the development of the

duct is in harmony with the view that it represents a modified gill-slit. But, if this is so, the slit would seem to belong rather to the 'primary' than to the 'secondary' (definitive right) series; since the 'primary' slits are formed in the mid-ventral line before shifting to the right and finally to the left. The position of the external pore on the left might be taken as evidence of this homology.

TEXT-FIG. 10.



Diagrams illustrating the mode of folding off of glandular region from the wall of the gut. A, B, C, three successive stages; A, youngest, C, oldest. Inner views of portion of endodermal gut, *end*, and body-wall, *ep*; *a*, anterior, and *p*, posterior. *c*, cut surface of duct; *d*, diverticulum to form duct; *f*, uprising fold; *io*, persisting internal opening; *l*, dotted line indicating glandular tube now on right of gut.

There appears to be nothing in the development of an ordinary gill-slit corresponding to the folding off of the glandular tube.

SUMMARY AND CONCLUSION.

In the account given above of the development of the club-shaped gland in *Amphioxus* several points are particularly to be noted. (1) The glandular part of the tube arises from a thickening of the endoderm on the right side of the gut. (2) This thickening does not extend across the mid-ventral line on to the left side. (3) The duct to the exterior arises very early from the endodermal wall of the gut as a mid-ventral diverticulum which soon grows towards the left and fuses at its tip with the ectoderm at a point below the mouth where an external opening is pierced later. (4) The lumen of the duct is an exten-

sion of that of the gut, and its communication with the exterior is established at about the time when the mouth and first gill-slit are perforated. (5) The separation of the glandular region from the gut takes place by the formation on the inner surface of the thickening of a transverse groove whose edges meet ventrally in a fold passing to the left of the entrance to the developing duct. (6) By the uprising of this fold and closing over of the lateral edges of the groove the glandular tube is separated off from the gut, with which it remains in communication by means of a dorsal opening. (7) At no time is the glandular region entirely cut off from the gut as a closed sac. (8) The lumen of the gland is not secondarily hollowed out in the solid mass of cells of the original thickening, but is directly derived from the cavity of the gut. (9) The internal opening once delimited persists until the whole gland degenerates at metamorphosis, and the same is true of the external pore.

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March 8, 1930.

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The Hatching of Insects from the Egg, and the Appearance of Air in the Tracheal System.

By

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and

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(From the London School of Hygiene and Tropical Medicine.)

With 8 Text-figures.

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INTRODUCTION.

It has recently been shown (Wigglesworth, 1930) that the extension of air into the tracheal capillaries of insects is determined largely by the osmotic pressure of the tissue-fluids in which these capillaries are bathed; and it was suggested that osmotic pressure might also be the force involved in the initial filling of the tracheal system with air at the time of hatching from the egg. It was argued, and the argument need not be repeated here, that, unless the nature of the tracheal wall changes later, the fluid present in the tracheae up to the time of hatching is

probably not 'serum', as it is usually said to be, i.e. a fluid containing both crystalloids and colloids like the tissue-fluids, but either pure water, or a solution in water of substances which could pass through the walls of the tracheal capillaries. The hypothesis was put forward that this fluid was an ultra-filtrate from the tissue-fluids into the tracheal system, the filtration being brought about by the hydrostatic pressure within the developing egg; and that after hatching, the hydrostatic pressure being reduced, this fluid was absorbed into the tissues by osmosis. This idea was suggested by the statement of Frankenberg (1915) that the developing larva of *Corethra* appeared to be subjected to great pressure.

The present work was undertaken in the first instance with a view to proving this hypothesis; and it may be said at once that it has proved to be unfounded. For it will be shown that in many insects the tracheal system fills with air while the larva is still inside the egg. On the other hand, we have made a number of observations upon the first appearance of air in the tracheae of a variety of insects, a subject about which comparatively little is known, and these observations provide a basis for discussion of the problem.

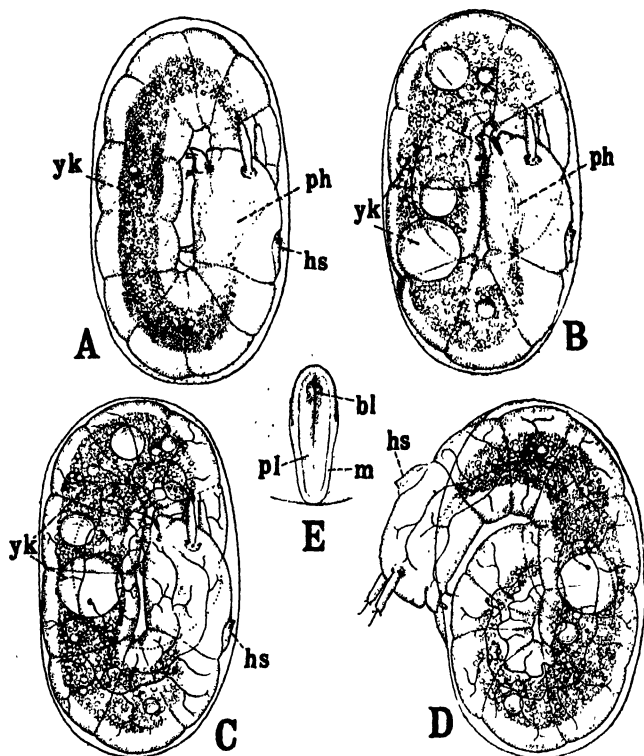
In the course of this work we have studied the mechanism of hatching in the insects dealt with. In this connexion we have added to the already long list of insects which possess 'hatching spines', and we have attempted some generalizations on the mechanism of emergence from the egg.

Hatching of Eggs of the Flea (*Ceratophyllus wickhami*) of the Grey Squirrel.

During its first instar the larva of the flea has on its head a powerful spine or egg-burster, figured, in the case of *Ceratophyllus wickhami*, by Sikes (1930). Although it has long been recognized (Künkel, 1873) that this structure is used by the larva to break through the chorion, its precise mode of action is not understood. Mitzmain (1910) observed the hatching of a single *Ceratophyllus acutus* larva, and gives a highly spirited account of the process; but our larvae followed the spectacular procedure there described only when the eggs

had been allowed to become too dry and therefore, judging by the many failures in hatching, were probably abnormal.

TEXT-FIG. 1.



Hatching of egg of flea. A, egg about 12 hours before hatching. B, larva swallowing the amniotic fluid: it is longer and stouter, the gut contains large droplets of fat, the hatching spine is beginning to project. C, larva dry on surface, tracheae filled with air. D, larva has moved round in shell and cut a longitudinal slit through which it is emerging. Note projection of hatching spine. *hs*, hatching spine; *ph* pharynx; *yk*, gut contents. E, hatching spine viewed from above, consisting of a median tooth (*bl*) and an elongated footplate (*pl*), surrounded by a thin membrane (*m*).

Text-fig. 1, A, shows the fully developed larva curled up in the egg, about twelve hours before hatching. It is floating freely

in the amniotic fluid and does not fill the shell completely. The spiracles and tracheae are visible but contain fluid. The gut is filled with the remains of the yolk, in the form of rather small globules of fat and much granular material.

At about this stage, pulsating movements in the head of the larva commence, and it is seen to be swallowing the amniotic fluid (Text-fig. 1, b). When this watery fluid is added to the fatty contents of the gut, the fat globules run together to form large droplets. At the same time the larva increases both in length and girth, until all the free fluid in the egg has been consumed, and the distended larva fills the shell completely. Packard (1894) observed that the flea embryo grew much longer just before hatching, but he did not detect the cause.

Next, the remaining amniotic fluid begins to evaporate and the surface of the larva becomes dry.¹ The spiracles are now exposed to the air, which soon enters them and extends into the tracheal system, moving rapidly along the main trunks, but very slowly along the finest branches, so that it may be half an hour or more before the smallest capillaries contain gas (Text-fig. 1, c.)

The larva now becomes more active and begins to crawl round inside the shell. It has been noted by Packard (1894) that the hatching spine of the young flea larva arises from the floor of a depression, so that it does not normally project above the surface of the head. But the spine springs from an elongated plate of chitin (Text-fig. 1, e) more rigid than the remainder of the head-capsule, and surrounded by a thinner membrane; so that, at the time of hatching, and for some hours afterwards (i.e. while the body is distended with fluid), this plate lies almost flush with the general level of the head. Consequently, the spine projects well above the surface (Text-fig. 1, d),² and, as the larva moves forward inside the egg, is pressed against the shell. Sooner or

¹ The drying of the larva may be recognized by the fact that the spiracles and the shagreened surface of the segments become more apparent, while the tracheae and other internal structures are less distinct.

² This state of affairs is figured without comment by Harms (1912). A similar arrangement is present also in the larvae of *Diptera Nematocera* (Edwards, 1919).

later the shell is pierced. But the larva continues to move forward, and the sharp front edge of the spine, acting in the manner of a tin-opener, cuts a longitudinal slit in the chorion. If all goes well, the slit extends about two-thirds the length of the egg, and the larva escapes, head foremost (Text-fig. 1, D). But sometimes, particularly if the egg has been kept too dry, the spine may slip out of the cut; and the larva then moves forward with the spine inside the shell again. Eventually, it may re-enter the old cut, or fresh cuts may be made, and sometimes the egg-shell may be slit in many places before the larva finally makes its escape. So far as we could judge, the inner membrane of the egg is cut through at the same time as the chorion.

It is clear that, as a general rule, the tracheal system of the flea larva fills with gas directly from the outside air, and not with gas dissolved in the tissue-fluids. We may suppose that, whatever the nature of the force which is tending to absorb the tracheal liquid into the tissues (see 'Discussion'), it is not sufficiently great to break the column of liquid while the larva is still bathed in the amniotic fluid. When the spiracles are open to the air, the liquid is absorbed without difficulty.

The absorbing force is active, evidently, for a considerable time before hatching. For if a larva is extracted from the egg some twelve or twenty-four hours before it would normally have hatched, as soon as the surface of the larva is dry, air enters the tracheal system in the same way as in the normal larva.

On the other hand, if the egg, containing a fully developed larva, is immersed in water before the air has entered the tracheal system, the larva may emerge as usual, though with fluid in the tracheae. As soon as the spiracles are exposed to the surface of the water, however, the tracheal system fills with air. Indeed, under these circumstances, the air enters with extreme rapidity, and may fill the system in less than thirty seconds; and this increased rate of filling suggests that, as a result of this delay, the absorbing force also has increased in strength. The significance of this will be discussed later.

We have never observed the tracheal system fill with gas while the egg or larva was under water, as happens normally in

insect larvae with closed tracheal systems.¹ But this may well be because the conditions were not entirely right for the experiment (cf. *Tenebrio*, below).

Hatching of Eggs of the Mealworm (*Tenebrio molitor*) (Coleoptera).

In most particulars the hatching of the mealworm resembles that of the flea larva, and it may be described very briefly.

Text-fig. 2, A, represents the egg of the mealworm about forty-eight hours before hatching. The larva is enclosed in a membrane, the first or 'embryonic' cuticle (Wheeler, 1889), which envelops each limb in a separate, unsegmented sac; a sac which is too small, so that the limb is greatly crumpled. The claws are pale. The larva is not coiled in the egg like the flea larva, but the segments are very short and thickened.

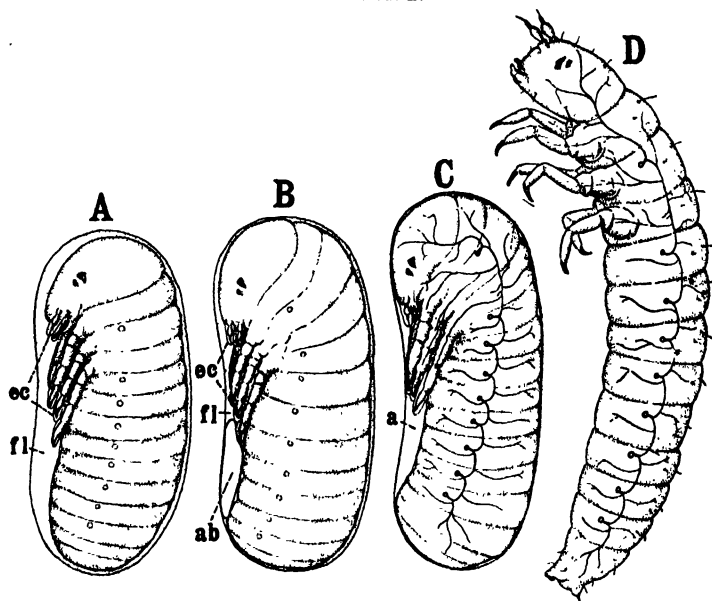
From time to time the larva swallows the amniotic fluid and, like the flea larva, comes to fill the shell more completely. The claws and mandibles slowly darken, and the body-segments become more elongated (Text-fig. 2, B), so that the head is flexed and the body somewhat curved. The outward form of the egg also is changed, becoming longer and thinner. Air enters the egg-shell, and occasionally some air may be swallowed by the larva; but this is not constant, and seems to play no essential part in the process of hatching. As the surface of the larva dries, air enters the spiracles and extends rapidly along the tracheae, though it often does not enter the finest branches in the legs until after hatching (Text-fig. 2, C).

The larva now becomes more active; perhaps it is invigorated by the improved supply of oxygen which follows the entry of air into the tracheal system. It forcibly extends the body, breaking off the head-end or the tail-end of the egg-shell, and so making its escape. The embryonic cuticle is shed shortly before, or at the time of rupturing the chorion. The larva does not swallow air after hatching, but it assumes its normal elongated form almost at once (Text-fig. 2, D), presumably by the

¹ *Corethra* (Krogh, 1911), Chironomidae (Pause, 1918; Keilin, 1924), Odonata (Calvert, 1898; Tillyard, 1916).

contraction of the transverse muscles of the body-wall. The mandibles play no part in opening the egg, and no hatching spines are present. Indeed, the chorion is so fragile that no such mechanism is needed.¹

TEXT-FIG. 2.



Hatching of egg of Mealworm. A, egg about 48 hours before hatching: larva, enclosed in embryonic cuticle (*ec*) does not fill the shell. B, after swallowing amniotic fluid (*fl*): larva fills shell, body elongated, head flexed; a bubble of air (*ab*) has appeared in shell. C, larva and egg much elongated; surface of larva dry, shell containing air (*a*); tracheae filled with air. D, larva immediately after hatching.

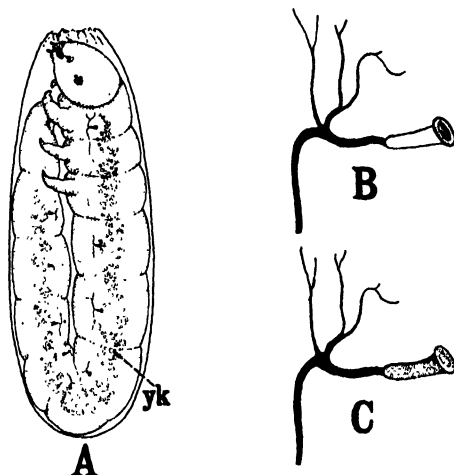
Thus, as in the flea larva, the tracheal system fills normally from the outside air. Like the flea larva, again, if the mealworm is removed from the egg before the amniotic fluid has disap-

¹ There is a row of short stout spines on either side of the clypeus, but these are present in subsequent instars, and so cannot be regarded as hatching spines in the ordinary sense. The same applies to the strong curved spines on the anal segment.

peared, the system fills with air as soon as the surface of the larva is dry.

On the other hand, if the egg is kept in water up to the time of hatching, provided it receives an adequate supply of air in

TEXT-FIG. 3.



Hatching of egg of Grain Moth. A, larva, with gut full of yolk (*yk*), biting its way out of the egg. Tracheae full of air. B, spiracle of larva still bathed in fluid; no air in the vestibule. C, spiracle when surface of larva dry; air extends to the outside.

solution (for example, if it is kept under a supported coverslip in a minute drop of water), the tracheae will fill with gas just as they do in aquatic larvae with the tracheal system closed.

Hatching of Eggs of the Grain Moth (*Sitotroga cerealella*).

The method of hatching in the Lepidoptera is well known, and the grain moth (*Sitotroga cerealella*) conforms to the general plan. When the larva is fully developed, it lies in an amniotic cavity entirely surrounded by a layer of yolk-cells. Shortly before hatching, it devours these cells, turning about in the shell with great activity.

Soon after these active movements have begun, the tracheae

are seen to have filled with air, although the larva is still bathed in fluid. The spiracles are closed and there is a clear space between the spiracles and the air-containing tracheae (Text-fig. 3, A and B.)

When the larva has consumed the yolk and the fluid contents of the egg, it proceeds to gnaw its way out (Text-fig. 3, A); the surface of the skin dries, the spiracles open, and the dark thread of air in the tracheae becomes continuous to the exterior. (Text-fig. 3, c.)

Hatching of Eggs of the Blow-fly (*Lucilia sericata*).

The embryology and hatching of *Musca* (= *Calliphora*) vomitoria have been described in detail by Weismann (1863), whose observations on the appearance of air in the tracheal system we can confirm; but since his observations on hatching were made on eggs from which the chorion had been removed, we can add some information on the method of hatching in the normal muscid egg.

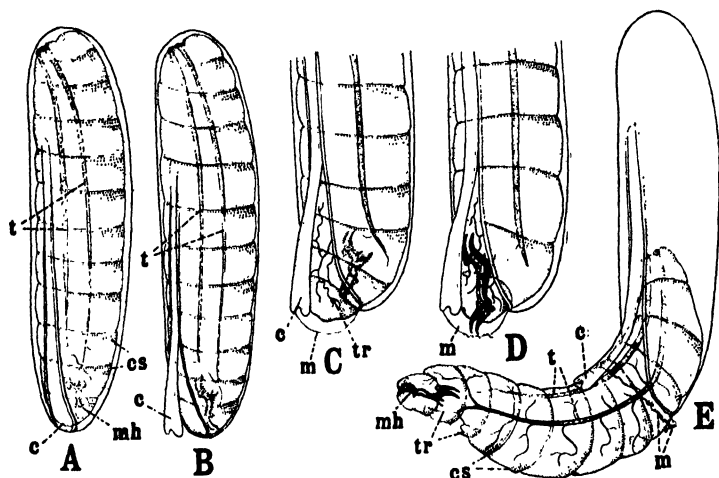
Along the dorsal surface of the egg, from the anterior pole backwards for about two-thirds of its length, the borders of the chorion are separated by a fissure, which is occupied by an elongated band tapering behind but broadening out in front (Text-fig. 4, A). We have found that this structure serves as a 'cap' precisely analogous to the cap on the eggs of Hemiptera or Anopleura.¹

A few hours before hatching, the rows of spines on the integument of the larva darken, so that it can be seen through the sculptured shell of the egg, moving actively about. Shortly afterwards the larger tracheal trunks fill with air (Text-fig. 4, A). They fill at a time when the egg still contains fluid, and, as noted by Weismann, they will fill even if the egg is immersed in water. We have found, however, that this occurs only if the egg is near the surface. If the eggs are kept under a coverslip and flooded

¹ Hewitt (1914) describes the larva of *Musca domestica* as escaping through a longitudinal slit in the chorion to one side of this dorsal band. Prell (1915) observed a more or less circular cap on the egg of the tachinid (*Parasetigena*), quite different from the elongated band of *Lucilia*.

with water, the larvae in those eggs which are more centrally placed, cease to move; presumably they are asphyxiated, and their tracheal systems do not fill with air. When exposed near the surface of the water, however, they revive, and gas soon appears in the tracheae.

TEXT-FIG. 4.



Hatching of egg of Blow-fly. A, egg a few hours before hatching; cap (c) closed; egg contains fluid, but tracheal trunks (*t*) filled with gas; cuticular spines (*cs*) and mouth-hooks (*mh*) visible. B, the Y-shaped cap (c) lifted. c, the vitelline membrane (*m*) bulging through the opening; tracheal branches (*tr*) contain gas. D, larva has perforated the membrane (*m*) with its mouth-hooks. E, larva escaping from egg; air is entering the finest tracheal branches; vitelline membrane (*m*) is seen as a cuff round the mouth of the egg.

As the time of hatching approaches, the larva becomes increasingly active. It works its mouth-parts in and out with a piston-like movement,¹ directing its blows mainly at the broad end of the 'cap'. Eventually this cap springs open and then appears (Text-fig. 4, B) as a Y-shaped flap attached by an elongated hinge. The larva continues its movements, and the vitelline membrane, distended with fluid, begins to protrude through the

¹ Mitzmain (1913) describes similar movements in *Tabanus* during hatching.

opening (Text-fig. 4, c). The larva now directs its energies against this membrane, scraping and striking with its mouth-hooks until the membrane is pierced (Text-fig. 4, d). It then works its way out, enlarging the split and probably swallowing much of the fluid with which it was surrounded in the egg. As the larva emerges (Text-fig. 4, e) air can be seen extending slowly along the fine tracheal capillaries. Sometimes the larva swallows air after emergence, but this is by no means invariable.

Hatching of Eggs of the Bed-bug (*Cimex lectularius*).

The mechanism of hatching in the bed-bug does not seem to have been described, though the process is essentially the same as that known in other Hemiptera, in the Neuroptera (Smith, 1920, 1922; Withycombe, 1924), and in the Psocidae (Peyerimhoff, 1901; Huie, 1916). In none of these insects, however, has the appearance of air in the tracheal system been considered.

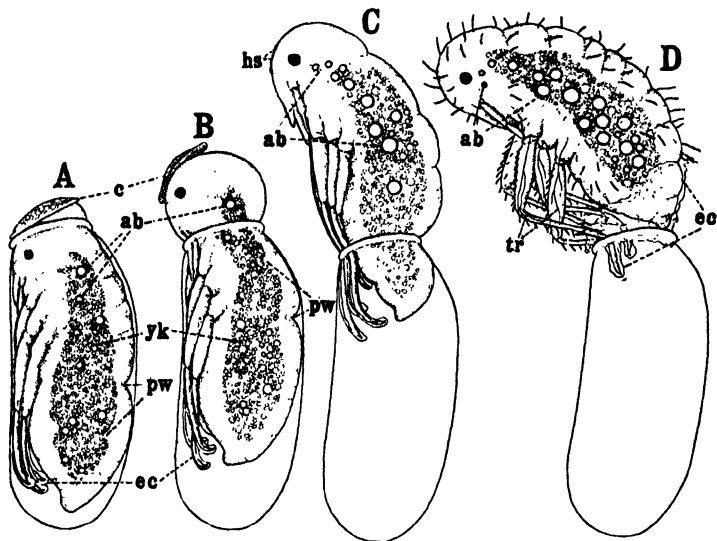
The larva of the bed-bug shows very little activity before hatching, and the sculpturing of the shell precludes any detailed observations upon it. But during the twenty-four hours before emergence the body of the insect comes to fill the egg more completely, the eyes moving forwards to the cap; the yolky contents of the gut become more apparent, the globules of fat becoming larger; and occasional pumping movements are visible in the head. It is probable, therefore, that the larva is swallowing the amniotic fluid during this period, like the other insects studied.

At the time of hatching, peristaltic, or rather anti-peristaltic movements of the gut, and similar movements of the abdominal wall, become very active, and drive the fluid contents of the insect into the region of the head. It is almost certainly this force which serves to dislodge the cap of the egg (Text-fig. 5, A); and the process is aided by the larva swallowing a certain amount of air, which increases the distension of the gut. As the cap is raised a small vesicle bulges through the orifice. This is composed of the escaping head and thorax, which are blown up like a bladder with the body-fluids. This bladder slowly enlarges as the larva squeezes its way through the constricted neck of the

egg (Text-fig. 5, B), and the process continues until only the tip of the abdomen remains inside (Text-fig. 5, C).

The insect, however, is still enclosed within its pre-larval skin or embryonic cuticle. It now begins once more to swallow large quantities of air (Text-fig. 5, C), distending the body more and more

TEXT-FIG. 5.



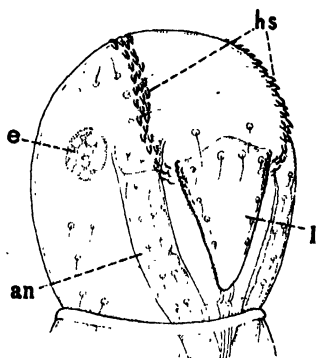
Hatching of egg of Bed-bug. A, cap of egg (c), being forced off; larva enclosed in embryonic cuticle (ec); abdomen and gut showing strong waves of peristalsis (pw) towards the head; gut contains globules of fat (yk) and a few small air-bubbles (ab). B, active peristalsis (pw) continues; head distended and bulging from the egg. C, larva, almost free from egg, has started to swallow air (ab) vigorously; hatching spines (hs) visible on front of head. D, embryonic cuticle (ec) has split and slipped backwards, allowing spines to stand erect; tracheae (tr) have filled with air; larva swallowing air (ab).

until the cuticle splits. The split appears over the top of the head and as the cuticle slips backwards, the bristles on the larva stand erect and the limbs and antennae become free (Text-fig. 5, D). During this process a layer of fluid can be seen between the larva and the cast skin; but on exposure, the surface of the

larva dries at once, and air enters the tracheal system for the first time. The air can be seen passing rapidly down the larger trunks, but more slowly along the finer branches, for example, in the distal portions of the legs.

When it has extricated itself from this first moult, the larva begins, for the third time, to swallow air most vigorously, until

TEXT-FIG. 6.



Head of Bed-bug, enclosed in embryonic cuticle, extruded from egg.
l, labrum with serrated margins; *hs*, hatching spines on embryonic cuticle; *e*, eye of larva; *an*, antenna in separate cuticular sheath.

the entire gut is enormously distended. Meanwhile it alternately flexes and extends its body, assuming its characteristic flattened form and increasing in size until it far exceeds that of the egg from which it came. Within an hour or so, all this air has disappeared.

There is another feature of the hatching mechanism which has not been mentioned so far. Between the embryonic cuticle or 'inner egg-membrane' and the chorion or 'outer egg-membrane' is a third layer, presumably the vitelline membrane, conveniently called by Speyer (1929) the 'middle egg-membrane'. Before it can escape from the egg, the larva must rupture this membrane, and to this end it has a series of hatching spines. These arise from the embryonic cuticle, and take the form of a file-like margin to the prominent labrum and, more particularly, a series of small teeth which are arranged in two divergent

tracts running downwards and backwards from the vertex of the head (Text-fig. 5, c, and Text-fig. 6). This hatching mechanism could not be observed in action; but judging by its structure it is probably actuated by slight flexion of the head. It is possible that it may play a part in the separation of the cap, but we have no evidence on this point. On the other hand, it is possible that these small, backwardly directed spines play no part in rupturing the membranes, but serve merely to prevent the head from slipping back into the egg as the cap is lifted.

A very similar hatching mechanism is present in the blood-sucking Reduviid bug, *Rhodnius prolixus*; but in this insect the file-like structure on the labrum is not present and the divergent tracts contain many more teeth.

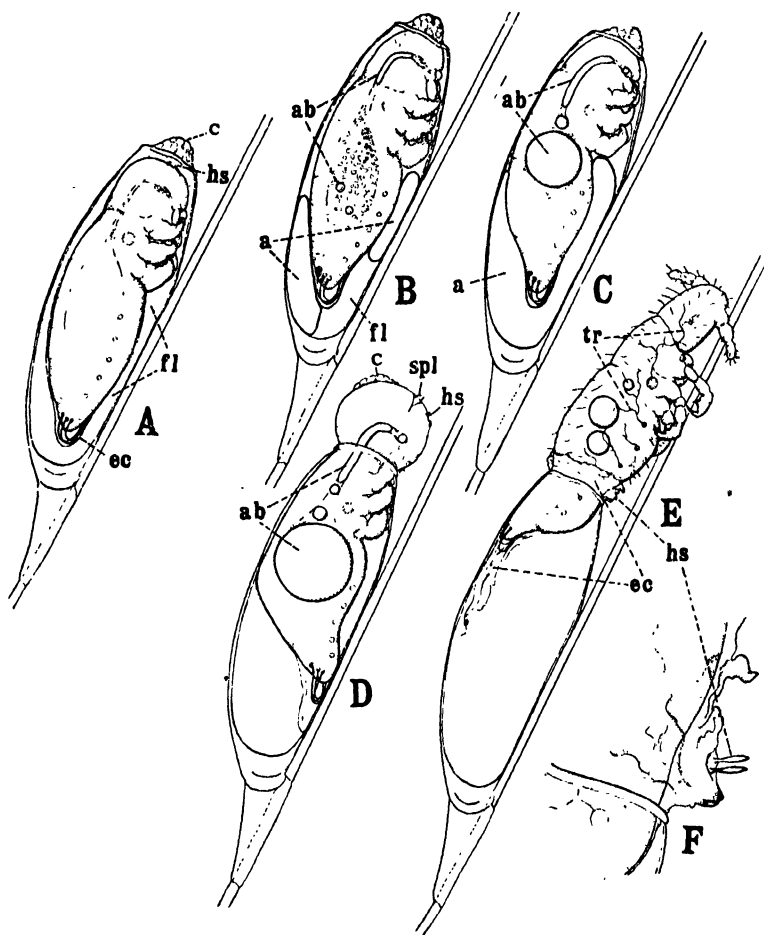
Hatching of Eggs of the Sucking-louse (*Polyplax serrata*) of the Mouse.

The hatching of the eggs of *Pediculus* has been described by Sikora (1915) and by Nuttall (1917), but these authors were not specially interested in the filling of the tracheal system. Further, the mechanism of hatching in *Polyplax* does not entirely agree with that given for *Pediculus*. Finally, we have found that all the genera of lice we have examined possess somewhat elaborate hatching spines, hitherto undescribed.

Text-fig. 7, A, shows the egg of *Polyplax* about twenty-four hours before hatching. The larva, enclosed within an embryonic cuticle which envelops each limb separately, is situated rather far back in the shell. The claws are pale. From time to time there are pulsating movements in the head, due to the insect swallowing the amniotic fluid. The hatching spines, which are shown in detail in Text-fig. 8, A, arise from a rigid area of the embryonic cuticle where it covers the front of the head. They consist of a pair of teeth, curved slightly upward; and, arising from the floor of a depression immediately above these teeth, a pair of lancet-shaped blades, side by side, directed straight forward.

During the last twenty-four hours of development the larva gradually moves forward, so that the blades of the hatching spines enter the cap of the egg. In so doing they must cut

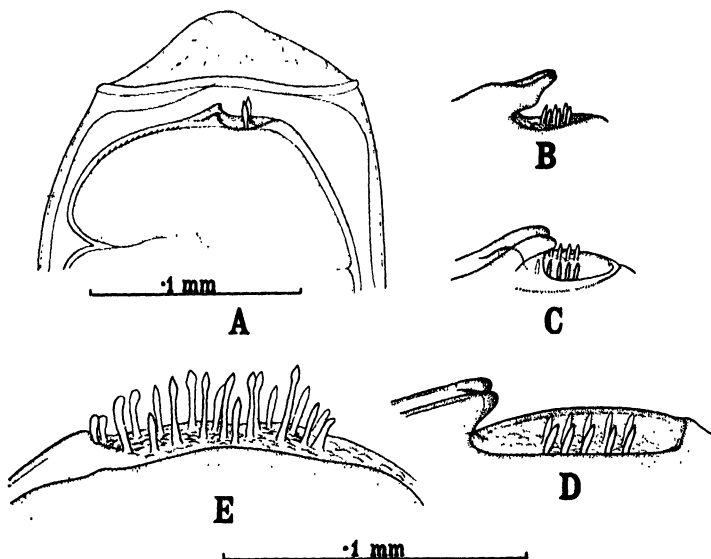
TEXT-FIG. 7.



Hatching of egg of Louse. A, egg about 24 hours before hatching, contains fluid (*fl*); larva, in embryonic cuticle (*ec*), well behind cap (*c*) of egg; hatching spines (*hs*) on front of head. B, larva has moved forwards; air (*a*) has appeared in shell; larva swallowing amniotic fluid and occasional bubbles of air (*ab*), body becoming distended. C, almost all fluid disappeared from shell; larva swallowing air (*ab*) very actively; gut distended with air. D, larva swallowing air and drawing up abdomen; cap (*c*) forced off and head bulging from the egg; embryonic cuticle has just split (*spl*). E, larva almost escaped from egg; tracheae (*tr*) filled with air; embryonic cuticle (*ec*) seen as a cuff bearing the hatching spines (*hs*). F, shows details of shed cuticle and hatching spines.

through the 'middle egg-membrane', and this is probably their main function. They now lie in the air-space within the cap. Meanwhile, one or more bubbles of air appear within the egg-shell, and as the larva continues to swallow the amniotic fluid

TEXT-FIG. 8.



Hatching spines on embryonic cuticle of various sucking-lice. A, *Polyplax serrata*; B, *Pedicinus rhesi*; C, *Phthirus inguinalis*; D, *Pediculus humanus*; E, *Haematopinus* sp.

and to press forwards to the front of the egg, these bubbles increase in size, until the space outside the embryonic cuticle is almost entirely filled with air. The larva continues to swallow, and, along with the fluid, it takes in bubbles of air (Text-fig. 7, B). Sikora and Nuttall state that in *Pediculus* this air is passed out through the rectum, accumulating behind the larva and so driving it from the egg. We have never seen this happen in *Polyplax*; indeed, seeing that the gut by this time is distended with watery fluid and fat-droplets, it would obviously require a very nice judgement to enable the insect to pass the

air while retaining the fluid. The bubbles which reach the stomach dissolve very rapidly and disappear. The oxygen they contain is doubtless used for respiration by the tissues of the larva. This will leave nitrogen with a high partial pressure, and, when the bubbles are small, under great tension, so that it will be quickly driven into solution.

Just before hatching, the larva begins to swallow air with great vigour, so that a large bubble accumulates in the gut (Text-fig. 7, c). At the same time it contracts the abdomen, and the increased pressure which results forces off the cap of the egg, and a little vesicle bulges out (Text-fig. 7, d). The swallowing of air and the contractions of the body continue, until the embryonic cuticle splits a little above the position of the hatching spines. The skin slips back, so that the hatching spines can be found attached to the cast skin at the mouth of the empty shell (Text-fig. 7, e).

The larva now works its way out of the cuticle and the egg, and as soon as the spiracles are exposed to the air, the tracheal system fills. (Text-fig. 7, e).

Text-fig. 8 shows the structure of the hatching spines in some other genera of lice. It is not necessary to describe each of these separately, but it will be noted that the general plan is alike in all, and that the number of blades is more or less in proportion to the size of the egg; ranging from a single pair in *Polyplax* to five pairs in *Phthirus* and *Pediculus* and about nine or ten pairs, irregularly arranged, in a large species of *Hæmatopinus*. The chief function of the lancet-shaped blades is probably, in all cases, to cut through the 'vitelline membrane', but the pair of teeth below them may perhaps assist in forcing off the cap of the egg.

DISCUSSION.

The Mechanism of Emergence from the Egg.

Heymons (1926) has reviewed the mechanism of hatching of insects from the egg, and van Emden (1925) has brought together all the examples of hatching spines which are known among insects, and has discussed their mode of action. The general conclusion reached by Heymons is that the chief force

which ruptures the shell is the internal pressure due to the growth of the embryo; and that the function of the various hatching mechanisms, whether they be 'egg-teeth' (Pentatomidae, &c.), hard plates (Sphodromantis, Mantidae), or soft distensible structures (Acridiidae), is merely to concentrate this increased pressure in one spot, and so cut through the chorion (e.g. Carabidae, Verhoeff, 1917) or to force off the cap of the egg (e.g. Pentatomidae). He compares the process with ecdysis, but claims that it differs therefrom in that the insect in the egg is never known to take up the outside air, either through the mouth or into the tracheae. Nor are any cases known in which the pressure is increased by the separation of gas from the tissues of the insect into the tracheal system. Thus, he says, important mechanisms for increasing the internal pressure are not available.

The observations recorded in the present paper allow some of these conclusions to be modified. In the first place, we have obtained no evidence that the growth of the embryo results in an increased pressure within the egg. In fact the reverse is probably the case. In *Cimex*, for example, a spoon-shaped depression often appears on either side of the egg a few days before hatching, and Nuttall (1917) records the same in *Pediculus*. The contracted form of the mealworm embryo gives the impression of its being under great pressure (cf. Frankenberg (1915) on *Corethra*), but even up to the time of hatching, the egg can be deformed by the slightest force; and the same applies to the egg of the flea.

The embryo certainly increases in size very rapidly before hatching. But it does so (*Ceratophyllus*, *Tenebrio*, *Cimex*, *Polyplax*) by swallowing the amniotic fluid. This, of course, will not increase the pressure in the egg, but it will give the insect a better purchase for its operations against the chorion. Although it has seldom been recognized before, the swallowing of the amniotic fluid before hatching is probably very common among insects. Thus Balfour-Browné records it in *Hydrobius* (1911) and *Dytiscus* (1913), Nuttall (1917) observed pumping movements in the head of *Pediculus* the day before hatching, and Huie (1916) saw this in *Steno-*

psocus. Tillyard (1915) describes what he calls the 'cephalic heart' in the embryo of the dragon-fly, but concludes in a later paper (Tillyard, 1916) that this is a 'temporary modification of the oesophagus'; and the movements described by Verhoeff (1921) in the embryo of *Carabus*, and associated with disappearance of the fluid in the egg, are almost certainly swallowing movements.

In every case the force employed to break open the egg appears to be muscular. Even where the insect is apparently motionless (*Cimex*) and seems to be impelled from the egg by some invisible force, there are peristaltic movements in the gut or abdominal wall which drive the body-fluids into the head (cf. Tillyard, 1916; Smith, 1920, 1922; Withycombe, 1924; Speyer, 1929), causing this to act in the same manner as the cervical ampulla of the acridiid larva (Künkel d'Herculais, 1890 *a*) or the ptilinum of the muscid imago. Voss (1911) has described a special musculature in the embryo of *Gryllus domesticus* which performs this function alone, and then degenerates.

The swallowing of air may also play an important part in the emergence of the larva. It may subserve three purposes. It may distend the larva while it is still inside the egg, the air for the purpose diffusing in through the shell, and so assist those movements of fluid by which the cap of the egg is dislodged (e.g. in *Cimex* and *Polyplax*, vide supra; in *Corydalis*, Smith, 1920). Then, in those cases in which the larva escapes from the egg while still enclosed in the embryonic cuticle, it may swallow air a second time, blowing itself up until this cuticle is forced to split (e.g. in *Cimex*, vide supra; in *Acridiidae*, Künkel d'Herculais, 1890 *b*; in *Psocidae*, Huie, 1916; in *Neuroptera*, Smith, 1922). Finally, the young larva may swallow air a third time, until its skin has stretched and it has attained a size much greater than the egg from which it came (e.g. *Cimex*, vide supra; *Benacus*, Needham (1907); *Psocidae*, Huie (1916); *Hydrobius*, Balfour-Browne (1911); and *Carabus*, Verhoeff (1921).

We have shown that in many cases the tracheal system fills with air while the larva is still inside the egg; but it is highly

improbable that this has any material effect upon the pressure within the shell.

As regards the mode of action of the hatching spines, we can add little to the suggestions of van Emden (1925) and Heymons (1926). The spine in the flea larva certainly acts as a blade which cuts a longitudinal slit in the chorion, like the spines in *Caraus* (Verhoeff, 1917) and the dorsal spine in *Psylla mali* (Speyer, 1929), and not merely as a wedge ('Drückkante') to split the shell. The newly described spines in *Cimex*, *Rhodnius*, and the *Anopleura*, would appear to be of use chiefly for breaking through the middle egg-membrane, unless, as already suggested, those in *Cimex* and *Rhodnius* serve to prevent the extruded head of the embryo from slipping back into the egg.

The First Appearance of Gas in the Tracheal System.

Most of the observations, in the past, on the first appearance of air in the tracheae of insects relate to the closed tracheal system and are reviewed by Keilin (1924). The only detailed observations on the filling of the open system with which we are familiar are those of Weismann (1863) on the larva of *Musca*, and Davies (1927) on *Sminthurus*.¹

The insects in which the process has now been observed fall into three groups: (i) those in which the surface of the larva dries while still in the egg, and the tracheal system fills, before hatching, from the outside air (*Ceratophyllus*, *Tenebrio*); (ii) those in which the larva at the time of emergence is enclosed in a cuticle which retains a layer of fluid beneath it, so that the air has access to the tracheae only when this 'embryonic cuticle' is shed, i.e. after hatching (*Cimex*, *Polyplox*); (iii) those in which the tracheal system fills with gas while the larva is still bathed in the fluid contents of the egg,

¹ Stadtmann-Averfeld (1923) describes a very improbable mechanism by which the tracheae of the mosquito larva fill with air. He supposes that muscular contractions squeeze all the fluid out of the tracheal system; and air is then taken in through the spiracle to replace it.

i.e. it fills like the closed tracheal system with the gases in solution (Sitotroga, Lucilia).

It is clear from the work of Weismann (1869) and Keilin (1924) that the fluid in the tracheal system is absorbed into the tissues; and it is probable that the mechanism of this absorption is the same in all cases.¹ Now the appearance of gas in the tracheae, especially in the larger trunks, is so rapid that it seems more likely that the absorption of fluid is due to some simple physical force than to secretory activity. It is probable, also, that the force which brings about this absorption is the same as that which normally keeps the tracheae more or less full of air. Now it has been shown (Wigglesworth, 1930) that the latter force is almost certainly the osmotic pressure of the tissue-fluids. The problem resolves itself, therefore, into two questions. How do the tracheae come to contain a fluid which can be absorbed by osmosis, i.e. a fluid whose constituents can pass through the walls of the tracheal capillaries; and is osmotic pressure a sufficient force to explain the filling of the system?

As regards the origin of the tracheal fluid, the notion which formed the basis of the present work, that the increasing hydrostatic pressure within the developing egg caused an ultrafiltration of the tissue-fluids through the walls of the tracheal tubes, has been shown to be fallacious. For an increased hydrostatic pressure does not exist (page 182), and in many insects the system fills with air before they leave the egg.

It was next thought that the fluid in the tracheae might be amniotic fluid. But it can readily be shown that the amniotic fluid of the mealworm egg contains proteins and salts like that of other animals.²

¹ That there is, indeed, no essential difference between the filling of the closed and the open systems is shown by the fact that, as described above, if the egg of the mealworm be kept in water, the tracheae may fill with the gases in solution.

² With the idea of changing the composition of the amniotic fluid or of the fluid in the tracheae, eggs of *Ceratophyllus* have been allowed to develop in aqueous solutions ranging from distilled water to one per cent. of sodium chloride; and the larvae have been allowed to hatch into these solutions with the tracheae still full of fluid. Prolonged exposure of the larva, after hatching, in any of these fluids may sometimes delay or entirely

If the tracheal liquid is not amniotic fluid, it must be produced within the tracheal system after closure of the spiracles. It has been shown by Weismann that the ingrowth of the ectoderm which becomes the trachea, has at first only a potential lumen; and that during development a cuticle is laid down and a lumen gradually forms. He assumes that as the lumen enlarges, fluid diffuses into it from the tissues. At a certain stage the cuticle of the larger trunks becomes impermeable to fluid. But the lumen goes on enlarging. Hence the space must be filled with gas, diffusing from the tissue-fluids; and in this way he explains the first appearance of gas in the tracheae. When development is complete, he supposes that the residual fluid is absorbed into the tissues through the finest tracheal tubes.

Before reading Weismann's work we had arrived at a somewhat similar hypothesis and obtained some evidence in its support. Mr. L. E. S. Eastham of Cambridge very kindly made some measurements of the tracheae of *Pieris* for us, at different stages of development. That they might be comparable, these measurements were made as near the spiracle as possible in each case. The results, which confirm those of Weismann on *Musca*, are shown in Table 1.

TABLE 1.

<i>Stage of Development.</i>	<i>Diameter of lumen in μ.</i>	<i>Diameter of trachea in μ.</i>
1. Tracheal ingrowth newly formed . . .	Capillary	12.0
2. A few days later	2.5	7.5
3. Shortly before hatching; cuticle formed	3.75	7.5
4. Newly-hatched larva	6.25	7.5

It will be seen that there is a progressive increase in the size of the lumen, with comparatively little change in the total diameter of the trachea. Assuming that the spiracles are closed, the fluid which occupies the lumen must have come from the tissues through the walls of the trachea. Now although these observations do not show whether this fluid is actively secreted prevent the subsequent filling of the tracheae, an effect which is unexplained; but apart from this the experiments gave no positive results.

by the epithelial cells of the tracheae, or whether it is a simple filtrate or dialysate, as assumed by Weismann; yet in either case its constituents might be expected to pass back again through the delicate walls of the tracheal capillaries. In the final stages of development the formative cells of the tracheae and tracheoles degenerate and practically disappear; so that the fluid in the lumen is directly subject to the osmotic pressure of the tissue-fluids; and if, as seems probable, the osmotic pressure increases about the time of hatching, conditions will be appropriate for the absorption of the fluid by osmosis.

Turning now to the question whether osmotic pressure is a sufficient force to explain the filling of the tracheal system, it is clear, from experiments on the larva of the mosquito (Wigglesworth, 1930), that the osmotic pressure of the tissue-fluids can absorb the liquid in the tracheae until air extends into the fine capillaries. There is no reason to doubt, therefore, that it is great enough to bring about the initial filling of the tracheal system when this is open to the outside air. But in order to fill the system when the insect is under water, or, what comes to the same thing, the closed tracheal system, it will be necessary first to overcome the cohesion of water before the column of fluid in the tracheae can be ruptured and the dissolved gas set free.

Now the experiments of Dixon (1914) have shown that, even with air in solution, the cohesion of water amounts to about 200 atmospheres pressure; whereas the available osmotic pressure in the tissue-fluids can only be of the order of 10 atmospheres (Wigglesworth, 1930). But in order to obtain this high figure for adhesion and cohesion of water, very special precautions had to be taken to remove all grease from the apparatus and to ensure that every part was thoroughly wetted. If this was not done, free gas appeared when the pressure was only slightly reduced; and Dixon admits that air-bubbles are common enough in the conducting tissues of the stems of plants, although the negative tension (due to the transpiration force) to which the sap is exposed is only between 5 and 20 atmospheres. In practice, therefore, even these relatively small reductions in pressure may bring about the liberation of gas; and this in spite of the fact that, since the conducting tubes of plants

imbibe water, they must exert a very strong adhesion to the sap. The tracheal intima of insects, on the other hand, has a notoriously low adhesion to water, so that it is not unreasonable to suppose that the osmotic pressure of the tissue-fluids will be sufficient to overcome this adhesion and effect the liberation of the gases in solution.¹

Keilin (1924), basing his argument on the high figures for the cohesion of water quoted above, considered that osmotic pressure could not account for the phenomenon; and he therefore invoked intramolecular changes in the protoplasm of the tissues, leading to an imbibition of water. But, as pointed out by Shull (1924) in the case of plants, it is difficult to see how imbibition could effect the removal of fluid from the closed tracheal tubes except through the secondary changes in osmotic pressure which it would induce.

If the insect is kept in water freed from gases, as was shown by Frankenberg (1915) on *Corethra*, the tracheal system does not fill. This was to be expected from the foregoing argument. But we are still faced with the problem why the tracheal system in some insects (e.g. *Sitotroga*, *Lucilia*) fills, like the closed system, with gases from solution; whereas in others (*Cimex*, *Polyplax*) it does not fill until after hatching.

It is notable that it is those larvae which show the greatest degree of activity within the egg whose tracheae normally fill with the gases in solution; and the most probable explanation is that it is the metabolites produced by this muscular activity which raise the osmotic pressure to the degree necessary to effect the liberation of gas. (Cf. Wigglesworth, 1930.)

Tillyard (1916) also noted that gas appeared in the tracheae of insects (dragon-fly larvae) at the time when muscular activity increased. He regarded the gas which first appeared as carbon dioxide, and explained its appearance at this time by its excessive accumulation in the active tissues. But in view of the great

¹ This point is well illustrated by a simple experiment devised by Dr. N. K. Adam (personal communication). Ordinary tap water is allowed to stand in two beakers, one thoroughly freed from grease with chromic acid, the other lined with paraffin wax. Bubbles of gas are soon liberated in the waxed beaker (at room temperature and atmospheric pressure) but not in the clean beaker. .

solubility of carbon dioxide and its rapid rate of diffusion in water,¹ the explanation given above is more probable.

Again, the closed tracheal system fills, as a rule, with great rapidity, whereas Davies (1927) noted that in *Sminthurus* the process of filling might take several days. He attributed this slow rate to the fact that the air enters through a single pair of spiracles, but a more likely explanation is that the osmotic pressure in the tissue-fluids of this insect is exceptionally low; whereas in the closed system the osmotic pressure must reach a relatively high value before filling can begin at all.

As to the nature of the gas which first appears, a subject which has provoked much discussion, there is no reason to suppose that this is constant. Its composition will depend solely upon the partial pressures and the invasion coefficients of the various gases in solution in the tissue-fluids at the moment when filling begins.

SUMMARY.

Hatching spines are described in the bugs, *Cimex* and *Rhodnius*; and in the lice, *Polyplax*, *Pedicinus*, *Pediculus*, *Phthirus*, and *Haematopinus*. In all these insects the spines occur on the embryonic cuticle which is shed at the time of hatching.

The mechanism of hatching is described in the following insects: the flea (*Ceratophyllus*), the mealworm (*Tenebrio*), the grain moth (*Sitotroga*), the blow-fly (*Lucilia*), the bed-bug (*Cimex*), and the sucking-louse (*Polyplax*).

In the light of these, and other observations in the literature, the general mechanism of the hatching of insect eggs is discussed.

The first appearance of air in the tracheae of these insects is described and the mechanism of the process considered. It is suggested that the fluid in the tracheae is absorbed by the osmotic pressure of the tissue-fluids, and that since osmotic pressure is increased by muscular activity, air appears earliest

¹ As soon as a bubble of gas has been liberated, the high coefficient of invasion of carbon dioxide between water and air will favour the accumulation of this gas more than the accumulation of oxygen or nitrogen.

in those insects which show the greatest activity while in the egg. It is argued that osmotic pressure will account also for the appearance of gas in the closed tracheal system.

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The Oogenesis of *Calanus finmarchicus*.

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With Plates 9 and 10, and 5 Text-figures.

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INTRODUCTION.

THE present investigation was undertaken with a view to working out the details of oogenesis in a Copepod, about which comparatively little is known from the modern cytological standpoint. *Calanus finmarchicus* was suggested by Mrs. R. C. Bisbee, of the Department of Zoology, University of Liverpool, as a suitable subject; it is easily obtainable in quantity and has relatively large eggs; moreover the nucleolus in its behaviour throughout the growth of the egg presents certain very interesting features.

The work was carried out partly in the Department of Biology, University College of Swansea, and partly in the Department of Zoology in the University of Edinburgh.

MATERIAL AND METHODS.

The specimens of *Calanus finmarchicus* (Gunnerus, 1770) used in the present investigation were obtained mainly from Plymouth, but also from the Marine Laboratories at Port Erin, Isle of Man, and at Millport, Butheshire.

The animals were examined at intervals of about fourteen days during the greater part of two years.

Some difficulty was experienced in obtaining satisfactory fixation of the ovary. Dissecting out the ovary before fixation was not found practicable but by cutting off the abdomen at the first joint before immersing in the fixing fluid, rapid penetration of the fixative was obtained with improved fixation of the delicate posterior end of the ovary.

Flemming-without-acetic acid followed by iron haematoxylin (4 or 5 hours in iron alum and overnight in 0.5 per cent. haematoxylin) generally gave the best results, but Bouin's fluid and corrosive sublimate were found excellent as nuclear fixatives. The Champy-Kull method gave good results in the older oocytes approaching maturation.

For the study of the mitochondria the methods of Kolatschev, Nassonov ('Microtomist's Vade-Mecum', 9th edition, page 345), and Champy-Kull were used. Flemming-without-acetic acid followed by iron haematoxylin was particularly successful in older oocytes.

For the study of the Golgi apparatus Da Fano's method gave the best results. The methods of Nassonov, Kolatschev, Kopsch, and Mann-Kopsch were tried without success.

The author acknowledges her indebtedness to Dr. F. A. Mocke-ridge of the University College, Swansea, where the work was begun, to Professor J. H. Ashworth, of the University of Edinburgh, to Professor W. J. Dakin, and Mrs. R. C. Bisbee, of the University of Liverpool, and Mr. L. A. Harvey, of the University of Edinburgh, for much helpful advice, and to the Committee of the Earl of Moray Endowment of the University of Edinburgh for a grant in aid of expenses.

LITERATURE.

Although the Copepoda as a group have been widely studied in the past, comparatively little is known of the details of their gametogenesis from the modern cytological standpoint. Prior to the development of modern cytological methods attention was directed almost exclusively to the nucleus and its chromosome content. The chromosome numbers for thirty-five species of Copepods are recorded in the 'Tabulae Biologicae 4' (1927). The majority of the numbers given are for members of the genus *Cyclops* but some species of *Gymnoplea* are included. Among the *Gymnoplea*, with the exception of a Japanese form *Diaptomus* sp. Ishikawa (1891) and *Diaptomus coeruleus* (Amma, 1911) the haploid number of chromosomes present in the female is either sixteen or seventeen, sixteen being the more usual number.

McClendon (1906, 1910) and Kornhauser (1915) both working upon parasitic copepods described the formation of ring-shaped double chromosomes by parasyn-desis in a way which appears similar to that recorded for *Calanus finmarchicus* in the present investigation.

Matscheck (1910) in a paper upon growth and development of copepod eggs recorded fragmentation of the nucleolus prior to the maturation divisions of the egg. This author also found formation of yolk in the half-grown oocytes and suggested a possible secretory function of the nucleolus. The more recent work of Ludford (1922, 1924) upon the morphology and physiology of the nucleolus provides strong evidence in support of this view. Gardiner (1927) working upon *Limulus polyphemus* has suggested a very specialized secretory function for the nucleolus in the transport of phosphorus to the cytosome.

The study of *Calanus finmarchicus* affords strong evidence for suggesting that the mitochondria play an important part in yolk-formation. A close relationship between the mitochondria and yolk-formation has been recorded in the eggs of various animals by recent workers (Harvey, 1926; King, 1926; and Gardiner, 1917, and others).

GENERAL ACCOUNT.

The ovary is single and median and is situated dorsal to the alimentary canal. It extends about two-thirds of the entire length of the thorax and tapers to a blunt point at its posterior end. From the anterior end of the ovary paired oviducts arise and run forward as wide thin walled tubes. These lie parallel to each other, dorsal to the alimentary canal after leaving the ovary, but at the anterior end each oviduct bends ventrally and laterally from its fellow and runs posteriorly, ventral to the alimentary canal. The two oviducts open together on the first abdominal segment close to the opening of the spermathecae. It appears probable that fertilization takes place as the eggs leave the oviducal opening.

Three distinct zones can be observed in the ovary; (Text-fig. 1).

- (1) A multiplication zone situated in the posterior part of the ovary and consisting of small cells—oogonia—undergoing mitosis. One or two cells at the tip of the ovary are larger than the other cells of the multiplication zone, these are probably primordial germ cells.
- (2) A narrow zone in which many of the cells show leptotene, pachytene, and synapsis stages in division.
- (3) A broad zone which occupies the whole of the anterior end of the ovary and which contains oocytes in a progressive series of growth phases. The anterior end of the ovary passes almost imperceptibly into the oviducts in which the later growth phases of the oocytes take place.

Multiplication Zone.

The oogonia at the posterior end of the ovary are very closely packed. Two or three nucleoli are present in the resting stage of the oogonial nucleus (figs. 1 and 2, Pl. 9). Owing to some difficulty which was experienced in obtaining good fixation of this part of the ovary the nature of the oogonial nucleoli is not perfectly clear. It was observed that one nucleolus is usually conspicuously larger than the others. After fixation by chromosmium technique followed by staining with iron haematoxylin,

TEXT-FIG. 1.

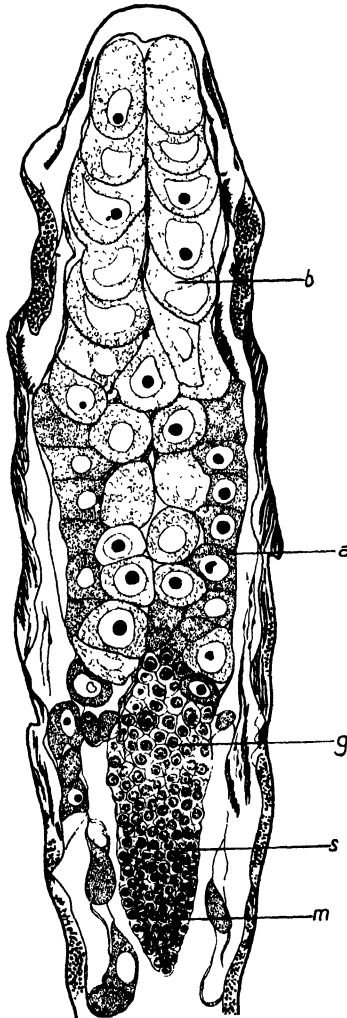


Diagram of longitudinal section through thorax showing zones of ovary, and arrangement of oocytes in the oviducts. *m*, multiplication zone; *s*, synapsis zone; *g*, growth zone; *a*, half-grown oocytes; *b*, older oocytes approaching maturation.

this larger nucleolus stains rather more lightly than the smaller ones. After Champy-Kull technique it stains a uniform amber colour and after Mann's methyl blue eosin, bright pink. It is a plasmosome and remains spherical in shape. The smaller nucleoli are irregular in outline and stain more deeply with iron haematoxylin. With Feulgen's 'Nuclealfarbung' method these nucleoli give a deep pink reaction: they are karyosomes.

The final oogonial mitoses, which actually mark the initiation of the growth phases of the oocyte, are visible in fixed and stained preparations immediately behind the synapsis zone of the ovary. The nucleoli disappear, or at least lose their staining properties, before the breakdown of the nuclear membrane: the chromatin network resolves itself into a very fine spireme thread which assumes a marked spiral arrangement within the nucleus. This thread breaks up into a number of long twisted chromosomes which shorten and thicken to form small rods. After the disappearance of the nuclear membrane these chromosomes, of which there appear to be about thirty-four, arrange themselves upon the equator of the spindle; metaphase, anaphase, and telophase follow in rapid succession. The chromosomes of this final oogonial mitosis swell in the telophase, forming a deeply staining mass at each pole of the spindle. The details of the events immediately following are rather obscured by this process, but the chromatin in the daughter cells formed by the division passes into the resting stage.

Synapsis Zone.

The resting stage of the nucleus of the early oocyte is of very short duration. Soon after the formation of the oocytes the nuclear network aggregates into masses which finally become drawn out to form a thread similar in appearance to the spireme thread of the early prophase nucleus of the oogonia. This thread breaks up into a large number of parts which become twisted and are extremely difficult to count in consequence. The chromatin then contracts to one pole of the nucleus and there forms a tangled, deeply staining knot. From this knot thick looped threads are seen projecting into the centre of the nucleus. Although no doubling of the threads was observed in the

leptotene stage, presumably this knot represents a synizesis figure in which the formation of bivalent chromosomes is taking place (fig. 4, Pl. 9). The bivalent chromosomes do not appear until a much later stage, the entire growth period of the oocyte intervening between the initiation and completion of the first maturation division. The synizesis knot, after a period of condensation in which it appears as a mass of chromatin at one pole of the nucleus, finally separates out to form a thread which closely resembles a spireme. This thread is much twisted upon it itself and surrounds the nucleolus when this latter re-forms. The oocytes remain in this condition throughout the subsequent growth stages.

Growth Zone.

In the very young oocytes at the beginning of the growth stage, a large central nucleus is present surrounded by a thin layer of rather flocculent cytoplasm. One or two nucleoli are present, one of which is a large plasmosome. The karyosomes at this stage stain a decided purplish pink when treated by Feulgen's method.

In slightly older oocytes only one large nucleolus is present which appears to be formed by the fusion of plasmosome and karyosomes and is therefore an amphinucleolus. Preparations made by Feulgen's method at this stage show a large clear body in the centre of the nucleus with a smaller pinkish body deeply embedded in its surface. This is interpreted as a stage in the fusion process of the plasmosome and karyosome. Preparations stained by the Champy-Kull method show the compound nucleolus as an amber-coloured body containing in its centre a varying number of highly refractive vesicles; these appear to be an intense bluish-green in colour. While this colour may be due to refraction, and the blue stain of the Champy-Kull method is a somewhat capricious one and cannot be taken as proving the presence of chromatin in the nucleolus, it appears probable from its mode of origin that chromatin is present in it at this stage.

The time of egg-laying varies considerably in different localities and is prolonged over a considerable period. Copepods from the west coast of Scotland had shed their eggs in most cases by the

beginning of June. Those from the south of England and from the Isle of Man were later and in some cases showed eggs in a very immature condition in mid-June.

THE NUCLEUS, NUCLEOLUS, AND NUCLEOLAR EXTRUSION.

Oogonia and young Oocytes.

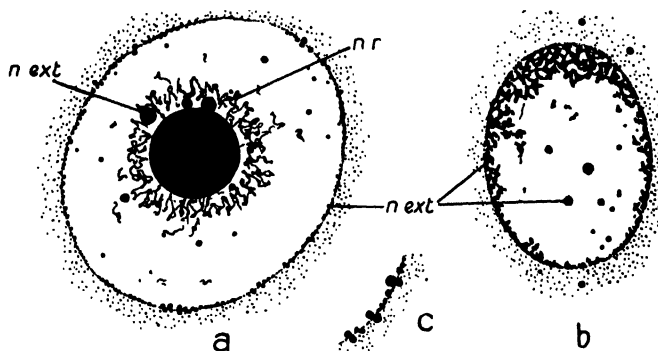
In the oogonia and very young oocytes the nucleus, which is centrally situated, occupies the greater part of the cell and a well-marked nuclear membrane is visible. The nucleoplasm stains a pale uniform grey with iron haematoxylin. The chromatin is aggregated into a number of irregular, deeply staining masses which lie close to the nuclear membrane, thus leaving a clear space in the centre of the nucleus in which the plasmosome lies. The karyosome is situated at one side of the nucleus in the oogonia and very young oocytes. It is frequently very irregular in shape and small, consequently it is easily confused with the chromatin masses round the periphery of the nucleus (fig. 3, Pl. 9). In preparations which were overstained with haematoxylin it retained the black stain more deeply than the surrounding chromatin. In slightly older oocytes the karyosome takes up a more central position and approximates towards the plasmosome, with which it finally fuses. Whether the other karyosomes when present fuse with the plasmosome or are used in chromosome formation it is impossible to say from the evidence available. No preparations were seen in which more than one karyosome appeared in the process of fusion with the plasmosome. In the older oocytes in which nucleolar extrusion was beginning only one nucleolus is present; this stains a uniform dark grey with iron haematoxylin and amber yellow with one or two highly refractive blue-green vesicles in the centre with Champy-Kull.

Nucleolar Extrusion.

Nucleolar extrusion takes place very rapidly throughout the early growth phases of the oocyte in *Calanus*. Portions of nucleolar material varying in size from minute spheres no larger than the mitochondria, to spheres which are as large as the fully formed yolk-globules, are extruded from the surface of the

nucleolus over the whole of its area. Although large spheres are seen in many preparations lying upon the surface of the nucleolus (Text-fig. 2 *a*), the nucleolus is not distorted and there is no indication that these extrusions arise by being pinched off. The nucleolar extrusions lying against the nuclear membrane are usually much smaller than those which are seen on or near the surface of the nucleolus; this suggests that the larger extrusions

TEXT-FIG. 2.



- a. Nucleus of half-grown oocyte showing nucleolar extrusions upon the surface of the nucleolus in the form of small spheres.
- b. Nucleus of half-grown oocyte showing nucleolar extrusions upon the surface of the membrane in the form of a network.
- c. Portion of the nuclear membrane showing coincidence of drops of nucleolar material on the inside and outside. *n ext*, nucleolar extrusions; *n r*, nuclear reticulum.

break up in their passage across the nucleus to the nuclear membrane. Since the extrusions are almost certainly of a liquid nature it is possible on the other hand that the drops are merely fixation effects, their size depending upon the concentration of extruded material in the region where they occur.

In very young oocytes the material which is extruded from the nucleolus forms a deeply staining mass which appears as a thick black line in the region of the nuclear membrane in preparations stained with iron haematoxylin (Text-fig. 4 *a*). This deposit shows more clearly in slightly older oocytes. In sections

3 to 5μ thick of material fixed with Flemming-without-acetic acid and stained by the long method in iron haematoxylin, a series of small black dots is seen closely apposed to the surface of the nuclear membrane. In some preparations the extruded material appears as a darkly staining reticulum lying upon the outer surface of the nuclear membrane (Text-fig. 2*b*). In sections from 8 to 10μ thick including portions of the surface of the nuclear membrane the extrusions sometimes appear as spherules of variable size lying outside the membrane but closely apposed to it. These are obviously fixation effects which signify the presence of a condensation of nucleolar material outside the nuclear membrane.

During the whole period of nucleolar extrusion there is present in the cytoplasm a varying number of deeply staining spheres which are in every way identical with the nucleolar extrusions inside the nuclear membrane. There can be no doubt that a large part of the nucleolar material is transported to the cytoplasm by the process of nucleolar emission. Although in young oocytes the majority of these spheres in the cytoplasm are found surrounding the nucleus, in the later stages of the growth of the oocyte they are sometimes found near the periphery of the cell. All these spheres in the cytoplasm pass gradually from a basophil to an acidophil condition, lose their staining properties and finally become invisible: presumably they are dissolved in the cytoplasm. In one or two preparations portions of the extruded material from the nucleolus appeared as though passing through the nuclear membrane into the cytoplasm (Text-fig. 2*c*). While this apparently supports the view held by some cytologists that nucleolar extrusions are passed through the nuclear membrane into the cytoplasm as individual bodies, a consideration of the whole process in *Calanus* suggests that this does not take place. In any case it seems highly improbable that bodies of the size of nucleolar extrusions could pass through the nuclear membrane without losing their identity or rupturing the membrane. It is probable that the appearance of these preparations is due to the coincidence of drops of nucleolar material closely apposed to the outside and the inside of the nuclear membrane at the moment of fixation. On the outer

surface of the nucleus there must be a considerable confluence of liquid nucleolar material which has passed through the membrane by a process of diffusion. From this semi-fluid perinuclear layer the nucleolar material appears to condense out as drops which migrate outwards into the cytoplasm, and finally become dissolved in its substance.

In the half-grown oocytes the nucleolus is no longer visible as a homogeneous mass in the centre of the nucleus, but is seen to consist of two distinct regions: a large central vacuole and a narrow outer rim. This appearance is constant with all fixatives and stains used, with the exception of Feulgen's method, which does not stain the nucleolus at this stage. In the younger oocytes the rim stains a uniform dark grey with iron haematoxylin, but in oocytes in which the process of nucleolar extrusion has been proceeding for a longer period a number of small vacuoles make their appearance in the rim (fig. 8, Pl. 9). This indicates a reorganization of the nucleolar material following nucleolar extrusion. Occasionally smaller vesicles can be seen in the core of the nucleolus which give it the appearance of an alveolar structure, but more often a faint granulation is all that is visible. This may be due to the coagulation of liquid substances in the central vacuole.

The Chromatin.

The nuclear reticulum of the half-grown oocytes is in the form of a fine network of much coiled interlacing threads. These threads are not of uniform thickness, but in places show small knots which probably represent aggregations of chromatin upon the linin network (fig. 8, Pl. 9). This network, although showing no trace of individual chromosomes, may be regarded as a diffuse and much modified diplotene stage. In all preparations observed the nuclear reticulum was seen contracted to a greater or lesser extent away from the edge of the nucleus and was most marked in the region immediately surrounding the nucleolus. This contraction does not necessarily indicate shrinkage due to fixation but may be an actual condition representing a re-concentration of the chromatin. For a short period the chromatin in the older oocytes approaching maturation is in the form

of a continuous thread. No indications of a double nature can be seen in this thread.

The maturation divisions of the egg usually take place in the ventral arms of the oviducts, although in some cases maturation appears to take place after the egg has left the oviduct. The early stages of the divisions of the ripe oocyte are very unstable, and the majority of the preparations examined during the maturation process showed either the metaphase or early anaphase position of the chromosomes upon the spindle. When the first maturation division has reached the metaphase there appears to be a distinct pause after which the final stages of the first division, the formation of the second spindle, and the completion of the second division are accomplished with great rapidity. It was impossible to say from the preparations examined whether the first polar body divides or not. The first maturation division, which is the true reduction division of the egg, enters upon its second phase with the breakdown of the chromatin reticulum to form bivalent chromosomes. During the period which intervenes between the first phase of the reduction division and the second phase, the entire growth of the oocyte takes place; changes occur in the cytoplasm and the greater part of the yolk is laid down. The nuclear membrane becomes constricted at one pole, the nucleus assuming an elongated pear shape as a result of the pressure of the eggs in the oviduct. At this time the nucleolus, which is comparatively quiescent during the later growth phases of the oocyte, enters upon a period of great activity. Quantities of nucleolar material are given off from the nucleolus and pass across the nucleoplasm into the cytoplasm. In fixed preparations this material appears in the form of spheres of considerable size which stain deeply with all basic dyes. After a short time in the cytoplasm the emissions lose their staining properties and become invisible (fig. 6, Pl. 9).

The chromatin reticulum during this process undergoes a second contraction into a tangled mass from which circular, bivalent chromosomes emerge. These are extremely small and aggregated together towards one side of the nucleus; their structure is by no means clear but in the earlier stages the ring

forms are seen to be deeply indented at opposite poles. From these bivalent ring-shaped chromosomes tetrads are formed by the appearance of a transverse constriction in each half of the ring; condensation takes place, and the tetrad is reduced to a compact body in which the four components are plainly visible.

The dissolution of the nuclear membrane begins at one pole of the nucleus and spreads rapidly. Immediately before its final disappearance, the nucleolus, which by this time is reduced to a small sphere, breaks up and passes into the cytoplasm. Throughout the period of the formation of the chromosomes a progressive loss in staining property is noticeable in the chromatin content of the cell. This is also true of the nucleolus, which finally shows a very similar staining reaction to the plasmosome of the early oocyte nucleus. The ring-shaped chromosomes when first formed stain faintly, but at a later stage when they are arranged upon the spindle they stain deeply with all basic dyes. Just before the disappearance of the nuclear membrane the group of seventeen tetrads is seen situated close to the nuclear membrane, usually at the opposite side of the nucleus to the disintegrating nucleolus (Text-fig. 8). With the disappearance of the membrane the tetrads pass out into the cytoplasm and take up their position on the equator of the maturation spindle.

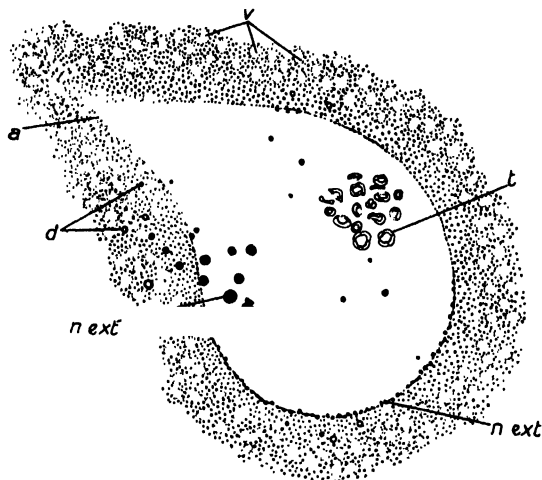
The Maturation Spindles.

The spindles are small truncated structures $10-13\mu$ in length (fig. 13, Pl. 10). Astral rays were not visible in any of the preparations examined but the longitudinal fibres were clearly visible. The spindles appear to be constructed of dense protoplasm which shows slight acidophil reactions. When arranged upon the equator of the spindle the chromosomes are very difficult to count owing to their small size and close proximity. When examined upon the equatorial plate the chromosomes sometimes appear V-shaped, converging towards the centre of the spindle while their free ends point outwards. This is probably due to tension at the point of attachment to the spindle fibres. After the halves of the tetrads have separated they move apart rapidly and the final stages of the division which was begun before the growth of the oocyte are completed.

The spindle of the second maturation division is formed at right angles to that of the first, and in rapid succession. Presumably a rotation of the second spindle occurs. Portions of first spindle are occasionally seen in the cytoplasm during the second division.

The second division is mitotic and separates the halves of the

TEXT-FIG. 3.



Nucleus of full-grown oocyte showing breakdown of nuclear membrane at *a*, tetrads (*t*) and final group of nucleolar extrusions. *d*, droplets; *n ext*, nucleolar extrusions; *v*, vacuoles.

monovalent chromosomes. Presumably the tetrads become so orientated upon the spindle that synaptic mates are separated from each other in the first division. The behaviour of all the chromosomes is identical. The polar bodies may be observed flattened against the surface of the egg by the pressure of the walls of the oviduct. They are very small, about 5 or 6 μ in diameter.

The chromatin in the nucleus is in a very unstable condition throughout the growth phases of the oocyte. Tests were made for chromatin by Feulgen's method, with the one modification that sections were left for two hours in the fuchsin sulphurous acid, and afterwards washed very quickly in two changes of

SO₂ water before mounting. It was found that in the nuclei of the oogonia and very young oocytes the nuclear reticulum stained a distinct purplish pink. In at least two cases which showed the karyosome in process of fusion with the plasmosome, the karyosome alone was stained. Everything else in the cell was colourless. Throughout the remainder of the growth stages the oocyte showed no trace of colour when treated by this method, and the nuclear network was invisible. In cells which were undergoing maturation, however, the chromosomes upon the spindle were deeply stained.

The affinity of the early oocyte karyosome for the stain coupled with the results obtained with the Champy-Kull method strongly suggests the presence of chromatin in the amphinucleolus during the early part of its history. There is no positive evidence for believing that chromatin is extruded from the nucleolus, but on the other hand the nucleoli of the more mature oocytes do not show any positive chromatin reaction. It is possible that the nucleolus acts as a reservoir for nucleic acid during the growth stages of the oocyte and that this is released previous to the formation of the chromosomes.

THE MITOCHONDRIA.

It was found that in the stages oogonia to oocytes the mitochondria were progressively more resistant to acetic acid and were in no cases completely destroyed by it. A marked resistance to acetic acid is not uncommon in the mitochondria of germ cells. It has been recorded by Nath (1926) for the scorpion *Palamnaeus*.

Structure and Distribution of the Mitochondria.

The mitochondria are present in the oogonia and very young oocytes in the form of a cap of mitochondrial material situated at one pole of the cell and closely adpressed to the nuclear membrane (fig. 2, Pl. 9). The cap is small and compact, and has clearly defined edges; it stains a dark uniform grey with iron haematoxylin following Flemming-without-acetic fixation, and bright pink with Champy-Kull. Occasionally the mitochondrial

cap appears in the form of several isolated masses, generally situated at one pole of the cell. It is probable that these slight variations in number and form of the mitochondrial masses are due to varying degrees of coalescence either before or at fixation. No individual mitochondria are visible at this stage.

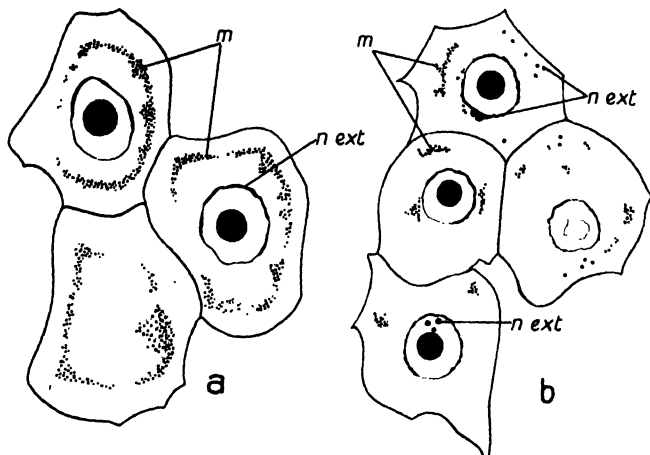
As growth of the oocyte proceeds the cap breaks up and gradually spreads out, moving as it does so away from the nuclear membrane into the cytoplasm. The amount of mitochondrial material increases considerably during this spreading out, but it is impossible to be certain whether the individual elements which compose the mass arise *de novo* in the cytoplasm or by the division of pre-existing mitochondria. One or two preparations showed figures which might be interpreted as division stages of the mitochondria and this correlated with the fact that the new masses of mitochondria always arise near the older ones makes it appear probable that multiplication does take place in this way.

In oocytes measuring 20–30 μ in diameter, the mitochondria in the form of small spheres surround the nucleus and are situated about half way between the nuclear membrane and the edge of the cytoplasm (Text-fig. 4 *a*). In the younger oocytes gaps occur in this hollow sphere of mitochondria but in the older oocytes it is quite complete.

Champy-Kull preparations at this stage show the mitochondrial masses to be composed of a large number of vesicles of varying sizes; the largest of these probably represent aggregations of several mitochondrial elements. With iron haematoxylin following Flemming-without-acetic, the elements which constitute the mass of mitochondrial material vary in size and shape from small spheres to filamentous structures which again probably represent a degree of coalescence. In all iron haematoxylin preparations observed the mitochondria appear to be arranged in a zone of cytoplasm which is darker and rather more flocculent in appearance than the surrounding medium. This appearance was observed to a lesser degree in preparations stained by the Champy-Kull method, but was not visible in material fixed in Nessonov or Kolatshev solutions which are specific for the mitochondria. While this cloud in the cytoplasm may be entirely

due to imperfect fixation of the mitochondria, it is also possible that it represents an accumulation of other substances which are present in the cytoplasm and come into relation with the mitochondria at this time. Nucleolar extrusion is very marked during the early growth phases of the oocyte and it is suggested that

TEXT-FIG. 4.



- a. Young oocytes showing mitochondrial ring and nucleolar material lying on the nuclear membrane. b. Oocytes after dispersal of mitochondrial ring (magnification about half that of a). *m*, mitochondria; *n ext*, nucleolar extrusions.

this cloud may represent a concentration of dissolved nucleolar material in the region of the developing mitochondria.

Woltereck in his paper upon growth and development of Ostracod eggs (1898) observed in Cypris a cap of material outside the nuclear membrane in the young oocytes which stained darkly with haematoxylin. This he described as a 'yolk nucleus' which in later stages of its development spreads out in the cytoplasm, sometimes appearing as small flocculent masses and at other times showing distinct granules of deeply staining substance upon a uniformly grey background. These various stages correspond so closely with the history of the development and gradual spreading of the mitochondrial ring in *Calanus*

finmarchicus that it seems probable the 'yolk nucleus' of Woltereck's description and the mitochondrial cap are identical structures.

The individual mitochondria which are scattered in the cytoplasm at a later stage of development are much smaller than the vesicles and spheres, which probably represent groups of fused mitochondria.

Before the oocytes are half grown the mitochondria begin to spread out through the cytoplasm. The spreading-out process by which the mitochondria pass from a stage when they are aggregated round the nucleus to a stage when they are more or less evenly distributed throughout the cytoplasm, begins at one point in the ring. The visible sign of this dispersal is the appearance of a well-marked gap which is first seen in oocytes measuring about 30μ in diameter. In slightly older oocytes the ring is seen to have dispersed with the exception of certain aggregations of mitochondria which appear to be much more stable than the rest of the mitochondrial ring (Text-fig. 4 b).

Occasionally two or three smaller groups remain, but one is the more usual condition. In half-grown oocytes traces of this last aggregation of mitochondria are visible which stain as a dark grey irregular mass with iron haematoxylin. It is significant that in a number of preparations yolk-formation was seen to be in progress near the periphery of the cytoplasm in this region (fig. 5, Pl. 9).

In the older oocytes the individual mitochondria are visible as minute spherical structures; these stain dark grey with iron haematoxylin and pink with Champy-Kull; they are uniformly distributed throughout the cytoplasm. There is a tendency for individual mitochondria to aggregate in groups of four and five, but no fusion takes place in the dispersed condition (fig. 10, Pl. 10).

Shortly after the dispersal of the mitochondria yolk-formation begins. The mitochondria swell and lose some of their staining properties. In their place small yolk-droplets appear which are at first arranged in small groups but which finally become scattered throughout the cytoplasm, where they enlarge. (See section on yolk-formation.)

In the mature oocytes the entire cytoplasm is packed with yolk-droplets and no mitochondria are visible (fig. 7, Pl. 9).

YOLK-FORMATION.

Although superficially the condition which is found in *Calanus finmarchicus* appears to support the view that yolk is formed by the direct chemical transformation of the mitochondria, a consideration of the other processes observed in the cell during the period of vitellogenesis leads the author to favour the view that a number of other factors are equally involved.

The yolk which is present in the oocytes of *Calanus finmarchicus* appears to be homogeneous and non-fatty in composition. Generally speaking, yolk-formation is first observed in the half-grown oocytes before the final distribution of the mitochondria has taken place. The quantity of yolk present at this time and its position in the cell is subject to slight variation. It may take the form of a group of well-marked droplets, situated at one side of the cell, or a few small droplets irregularly scattered near the periphery. It is impossible to say with certainty that this early formed yolk has any direct relation to the mitochondrial masses still visible in the young oocyte, but subsequent events suggest that this is the case; furthermore, it has been observed that where a group of mitochondria remain in the cytoplasm, the yolk-droplets are more numerous in the region of the cytoplasm lying between this mitochondrial group and the periphery of the cell (fig. 5, Pl. 9).

At a later stage the mitochondria which are dispersed swell and stain much more deeply, finally becoming replaced in position by yolk-droplets (fig. 10, Pl. 10). This replacement of the mitochondria by yolk-droplets proceeds from the periphery of the cell inwards until the cytoplasm is packed with yolk. This is the condition found in the eggs which have undergone maturation and are situated near the posterior end of the oviducts.

While it is obvious in this case that the mitochondria are intimately connected with yolk-formation, it is impossible to say with certainty from the observed facts whether the scattered

mitochondria are directly transformed into yolk by a chemical change, or whether they serve as reservoirs for materials deposited in them at this time.

The period of most rapid yolk-formation appears to be that immediately preceding the breaking down of the nuclear membrane in the ripe oocyte. This period coincides with a rapid and final activity on the part of the nucleolus. During the formation of the chromosomes this splits into fragments, the parts passing out into the cytoplasm, where they are finally dissolved. It is significant that the most rapid period of yolk-formation in the egg of *Calanus* should coincide with this final period of nucleolar extrusion. If the nucleolar extrusions play any part in yolk-formation, and if, as was suggested, they come into relation with the mitochondrial ring during its formation, what appears to be a precocious formation of yolk in the region of the mitochondrial masses can be partially explained. In the section on the mitochondria certain cases were described in which the mitochondrial masses appeared to be surrounded by a darkly staining cloud in the cytoplasm. It was suggested that this cloud might indicate the presence of an accumulation of dissolved material in the region of the mitochondria and that this material is probably nucleolar in origin. In these cases yolk-droplets were frequently seen near the mitochondrial mass.

About the time when yolk makes its first appearance in the cell, changes were observed in the structure and staining reactions of the cytoplasm, which in the young oocytes is flocculent in appearance and oxyphil. Throughout the growth phases of the oocyte a gradual change from oxyphily to basophily has been observed, the cytoplasm at the same time becoming denser and more granular in appearance. In the older oocytes a return to a condition of secondary oxyphily takes place and the cytoplasm loses its granular appearance, becoming at first flocculent and later highly vacuolated. The beginning of vacuolation corresponds with the onset of yolk-formation (fig. 9, Pl. 9). The vacuoles in the cytoplasm are filled with a watery fluid which condenses out in some preparations as large drops which stain a greenish grey with iron haematoxylin and yellowish with Champy-Kull. When visible these drops are always seen in

association with yolk-droplets. It is possible that they represent accumulations of substances passing from the cytoplasm to the mitochondria during the formation of yolk therein (fig. 9, Pl. 9).

With the exception of one or two doubtful cases the Golgi elements have, so far, not been observed in the younger oocytes, and it is therefore impossible to say whether or not they play any part in yolk-formation. In the older oocytes no visible connexion was observed between individual Golgi elements and yolk-droplets, nor was their position in any way correlated with the region of yolk-formation in the cell.

It is suggested therefore, that yolk in *Calanus finmarchicus* is formed in the mitochondria by the transformation of part of their own substance and the deposition in them of substances derived from the nucleolus and the cytoplasm.

THE GOLGI APPARATUS.

In the oogonia and very young oocytes fixed with Flemming and stained with iron haematoxylin, deeply-staining spherical structures were seen closely pressed against the nuclear membrane in some preparations. Although adjacent to it, these structures appeared quite separate from the mitochondrial cap (fig. 2, Pl. 9). The close proximity of the mitochondrial cap in the oogonia and the presence of nucleolar emissions upon the surface of the membrane in the young oocytes render it difficult to be certain of the nature of these bodies. While they may represent smaller aggregations of mitochondria which have separated from the cap and tend, owing to their small size, to become spherical there are indications of a non-staining chromophobe centre and a deeply-staining chromophilic rim in one or two cases. In some other cases where this rim is not visible they are stained more deeply with iron haematoxylin than the adjacent mitochondrial masses. This staining reaction suggests the possibility that these structures are Golgi bodies. No trace of impregnation was found by silver nitrate or osmium tetroxide methods in oogonia or young oocytes to substantiate this view and all attempts to demonstrate the apparatus in the young cell by any other method have so far been unsuccessful.

The Golgi apparatus was first identified without doubt in oocytes measuring 40–50 μ in diameter. By Da Fano's method clear pictures were obtained which showed the apparatus in the form of black uneven granules of irregular shape, lying mainly towards one pole of the cell (fig. 15, Pl. 10).

In one or two cases a very heavy impregnation occurred, but it is unlikely that the whole of this signifies the presence of Golgi elements.

In half-grown oocytes the silver deposit is much lighter and the individual elements are scattered over a much larger area while still being mainly concentrated towards one side of the nucleus. In the mature oocytes the silver nitrate method shows a uniform distribution of the Golgi elements throughout the cytoplasm in the form of small bodies of irregular shape but smooth outline. No trace of a network could be distinguished (figs. 11 and 13, Pl. 10).

In two half-grown oocytes fixed in Flemming and stained with iron haematoxylin a structure of doubtful origin was seen lying in the cytoplasm close to the nuclear membrane. This structure, which was spherical when examined in section, appeared to consist of a clear chromophobe centre and a well-defined chromophile rim. Although lying close to the nucleus it had no connexion with it. The cytoplasm immediately surrounding this body stained rather more lightly than the cytoplasm in the rest of the cell. In staining reaction and in general appearance this structure was very like the spherical form of the Golgi apparatus seen in optical section when stained by this method, but it was unusually large for a Golgi body and furthermore was not found in any of the other oocytes of the same age treated by the same method. Its exact nature and origin remains a mystery (fig. 10, Pl. 10).

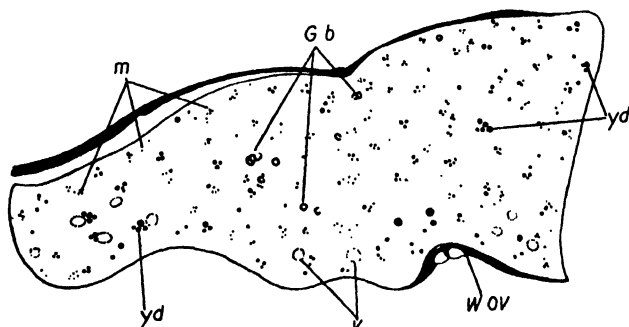
In the older oocytes at the onset of yolk-formation one or two preparations stained with iron haematoxylin showed the ring-shaped Golgi elements scattered in the cytoplasm. These were smaller than those found in the younger oocytes (Text-fig. 5).

The last stage of the Golgi apparatus, in which it occurs as scattered granules distributed throughout the cytoplasm of the ripe oocytes, may be termed the 'diffuse stage' in contrast to the

earlier 'complex stage' where it is concentrated in one part of the cell.

Careful examination failed to reveal any connexion between the developing yolk-droplets and the individual Golgi elements, nor was the position of the complex stage in the cell in any way related to regions where yolk-formation was taking place. The

TEXT-FIG. 5.



Oocyte at the beginning of yolk-formation showing Golgi bodies, scattered mitochondria, yolk-droplets, and beginning of vacuolation in the cytoplasm. *G b*, Golgi bodies; *v*, vacuoles; *w ov*, wall of oviduct; *y d*, yolk-droplets.

function of the Golgi apparatus in *Calanus* remains an open question.

DISCUSSION.

In the present investigation several points arise for discussion. These fall into two main groups; questions concerning the behaviour of the chromosomes and the process of nucleolar activity. Associated with the latter is the question of yolk-formation in the oocyte and the part played in the process by nucleolar emissions. The following points are dealt with under these headings.

The chromosomes. In the very young oocytes of *Calanus finmarchicus* a typical synzesis figure is formed, but no bivalent chromosomes emerge from this although presumably the bivalents are present in the synzesis knot. The bivalents appear for the first time after the growth of the oocyte

is completed and immediately prior to the first maturation division. During the entire growth phase no individual chromosomes are visible but a much twisted thread surrounds the nucleolus. Among the Amphibia¹ it is known that the deconcentration of the chromosomes proceeds so far that many, or all of them, are indistinguishable during the growth phases of the egg. In these extreme cases the germinal vesicle shows only an oxyphilic lightly-staining meshwork surrounding one or two nucleoli which presumably contain the entire basophilic content of the nucleus. In the disappearance of the individual chromosomes and the presence of the twisted thread surrounding the nucleolus the condition found in *Calanus* is comparable to this, but there is no evidence for believing that the nucleolus contains the entire basophilic content of the nucleus; on the contrary the thread surrounding it stains with basic stains if rather more lightly than the nucleolus. It appears probable that this network surrounding the nucleolus is in reality a much modified spireme formed from the deconcentrated chromosomes, which lose their individuality during the growth phases of the oocyte and reappear as ring-shaped bivalents at maturation.

The ring-shaped form of the chromosomes is very constant for the Copepoda. Many of the earlier workers who observed them believed them to be formed from chromosomes which had united by telosyndesis during the period at which the chromatin is massed at one pole of the early oocyte nucleus. Kornhauser (1915) observed these rings in the Copepoda but believed them to be formed from chromosomes which had united by parasyndesis in the early oocyte nucleus. He claimed that each of these chromosomes showed at an early stage a distinct transverse split which he supposed to be an integral part of the structure of the chromosome. No trace of such a split was visible in any of the chromosomes of *Calanus finmarchicus* and from the material examined it was impossible to say with any degree of certainty whether the bivalents were formed by

¹ Anura Oscar Schultze (1887), Carnoy and Lebrun (1879), King (1908). Urodela Born (1894), Carnoy and Lebrun (1878, 1879), Schmidt (1905), Jorgensen (1913), Stieve (1920).

telosyndesis or parasyndesis, though the latter appears more probable.

Nucleolar extrusion. Throughout the entire growth phase of the oocyte nucleolar activity is very marked in *Calanus finmarchicus*. The exact nature of the portions of nucleolar material which pass out into the cytoplasm and their ultimate fate is difficult to determine. The majority of the earlier workers upon the Copepoda failed to establish the passage of the nucleolar extrusions into the cytoplasm although they observed them within the nuclear membrane. Moroff (1909) figured for *Paracalanus parvus* fragmentation of the nucleolus and the presence in the cytoplasm of granular masses of material which he believed to be nucleolar in origin. These figures correspond so closely with those obtained for *Calanus finmarchicus* after Flemming and iron haematoxylin that there is little doubt the granular masses in the cytoplasm were mitochondrial in nature: in no cases were masses of nucleolar material seen in the cytoplasm in *Calanus*, having once condensed out from the surface of the nuclear membrane the extrusions appeared as scattered drops moving towards the periphery of the cell. There is absolutely no evidence that they pierce the membrane as whole bodies though this condition is reported by Nath and Mehta (1929) in the eggs of the Firefly. While it has not been possible to establish a definite periodicity in the behaviour of the nucleolus in *Calanus* there is evidence for believing that its activity is much more marked at certain stages of the growth of the oocyte than at others. In all the young oocytes examined, numerous nucleolar emissions of varying sizes were seen inside the nucleus, while outside the nuclear membrane an accumulation of nucleolar material was visible. This was much less marked in the older oocytes but immediately before maturation a second period of marked activity on the part of the nucleolus occurs. The first of these periods coincides with the stage at which the mitochondria are arranged in a ring surrounding the nucleus. It has already been suggested that the nucleolar emissions may come into relation with the developing mitochondria at this time and that the dark cloud in the cytoplasm surrounding them which is seen in some preparations may

consist of secretions from the cytoplasm and nucleolus. The second period of marked activity coincides with the deposition of yolk in the oocyte, the breakdown of the nuclear membrane, and the formation of the chromosomes. That part of the nucleolar substance is used up in the formation of the chromosomes is probable, but there is considerable evidence to show that the emissions which pass into the cytoplasm are in some way connected with yolk-formation.

The method of yolk-formation in the egg has been the subject of much recent research. Numerous records of the close relationship between mitochondria and yolk, and between Golgi apparatus and yolk are to be found. Many of these are reviewed in a recent paper by Hibbard (1928). Other cytologists have put forward the view that the nucleolus is directly concerned in yolk-formation. Nath and Mehta (1929) and Gresson (1929) have recorded the formation of yolk from nucleolar emissions in the cytoplasm. The formation of albuminous yolk from nucleolar emissions has been described in the cockroach by Hogben (1920) and in *Saccocirrus* by Gatenby (1922); other examples might be cited. In a paper on the oogenesis of *Limulus polyphemus* Gardiner (1927) proved the presence of substances rich in phosphorus in the nucleolus and suggested that the mechanism of nucleolar emission effects the transport of phosphorus from the nucleus to the cytoplasm. It appears probable that in *Calanus* one of the functions of nucleolar activity is to provide a means of transport by which substances used in the formation of yolk are passed from the nucleus into the cytoplasm.

SUMMARY.

Three regions can be recognized in the ovary: a multiplication zone containing oogonia undergoing mitosis, a synapsis zone containing the first formed oocytes in the prophases of the maturation division, and a growth zone containing oocytes in a series of growth phases with the nucleus in a 'resting condition'.

The oogonial nuclei contain two or three nucleoli—plasmosome and karyosomes. In the oocytes a single nucleolus is present; this is formed by the fusion of the plasmosome and at

least one karyosome and is therefore an amphinucleolus. The chromatin in the oogonia and young oocytes is arranged round the periphery of the nucleus and is aggregated in knots (pp. 196-200).

Nucleolar extrusion begins in the young oocyte and continues throughout the growth period. It is most marked in the young oocytes and in oocytes about to undergo maturation (pp. 200-3).

In the older oocytes the chromatin is in the form of a tangled thread surrounding the nucleolus. Immediately before maturation this condenses and circular chromosomes emerge: these form tetrads (pp. 203-5).

The mitochondria are present in the oogonia and very young oocytes in the form of a cap lying upon the surface of the nuclear membrane. The mitochondrial elements spread and multiply until they surround the nucleus as a ring; afterwards they disperse and are distributed evenly throughout the cytoplasm. They swell up and finally yolk-droplets appear in their place (pp. 207-11).

Yolk-formation usually begins in half-grown oocytes, but is sometimes earlier. The formation of yolk-droplets begins at the periphery of the cell and proceeds inwards. It is suggested that yolk is formed by transformation of the mitochondria and the deposition in them of substances derived from the cytoplasm and the nucleolus. The cytoplasm is flocculent in the young oocytes, granular in the half-grown oocytes, and filled with fluid vacuoles in mature oocytes. It passes from a primary condition of oxyphily to basophily and finally back to a secondary oxyphil condition in mature oocytes (pp. 211-13).

In the young oocytes deeply-staining spherical structures were seen adjacent to the mitochondrial cap. From their appearance it is possible these bodies represent the Golgi apparatus, but Da Fano fixation failed to demonstrate them. In half-grown oocytes the apparatus was visible in the complex condition at one side of the cell. As growth proceeds it passes from a complex to a diffuse condition and in mature oocytes the Golgi elements are uniformly distributed throughout the cytoplasm (pp. 213-15).

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EXPLANATION OF FIGURES

The figures are magnified about 500 diameters except where otherwise stated.

KEY TO LETTERING.

a, fusion of plasmosome and karyosome; *ch m*, chromatin mass; *chr*, chromatin; *chrom*, chromosomes; *d*, droplets; *e p*, equatorial plate; *G b*, Golgi bodies; *k*, karyosome; *lep st*, leptotene stage; *m*, mitochondria; *m c*, mitochondrial cap; *n* nucleus; *n b*, nuclear membrane; *n ext*, nucleolar extrusions; *n r*, nuclear reticulum; *p*, plasmosome; *sp*, spindle; *spir*, spireme; *syn st*, synapsis stage; *t*, tetrads; *v*, vacuole; *W OV*, wall of oviduct; *x*, cytoplasmic body; *y d*, yolk-droplets; *1st m sp*, first maturation spindle. (In description of Plate, below) C.K., Champy-Kull; Da F., Da Fano; E.H., Ehrlich's haematoxylin; F., Flemming; F.w.a., Flemming-without-acetic acid; I.H., Iron haematoxylin.

PLATE 9.

Fig. 1.—Oogonia from the tip of the ovary showing mitosis and resting stages. (Bouin, I.H.).

Fig. 2.—Oogonia showing mitochondrial cap and possible Golgi bodies. (Nassonov, I.H.).

Fig. 3.—Young oocytes showing spreading of the mitochondrial cap and Golgi bodies. (F.w.a., I.H.).

Fig. 4.—Oocytes from the synapsis zone showing synapsis and leptotene stages and mitochondrial cap. (F.w.a., I.H.).

Fig. 5.—Half-grown oocyte showing mitochondrial masses, nucleolar extrusion and precocious yolk-formation. (F.w.a., I.H.).

Fig. 6.—Nucleus of oocyte about to undergo maturation, showing ring-shaped chromosomes and final breaking up of the nucleolus. (C.K.).

Fig. 7.—Oocyte from posterior end of oviduct showing cytoplasm packed with yolk-droplets. (F.w.a., I.H.).

Fig. 8.—Young oocyte showing vacuolation in the rim of the nucleus and granular core, nuclear reticulum, and nucleolar extrusion. (F.w.a., I.H.).

Fig. 9.—Part of the cytoplasm of an oocyte before maturation showing yolk-formation, vacuolation of the cytoplasm, and chromophobe droplets at *d*. (F.w.a., I.H.).

PLATE 10.

Fig. 10.—Half-grown oocytes from anterior end of the oviduct showing nucleolar extrusion, beginning of yolk-formation and body of unknown origin in the cytoplasm at *x*. (F.w.a., I.H.).

Fig. 11.—Oocyte during first maturation division showing equatorial plate and diffuse condition of Golgi apparatus. (Da F., E.H.).

Fig. 12.—Young oocyte showing large central vacuole of nucleolus and Golgi bodies surrounding nucleus on one side. (Da F., E.H.).

Fig. 13.—Oocyte during first maturation division showing spindle with tetrads at metaphase, and diffuse condition of Golgi apparatus. (Da F., E.H.).

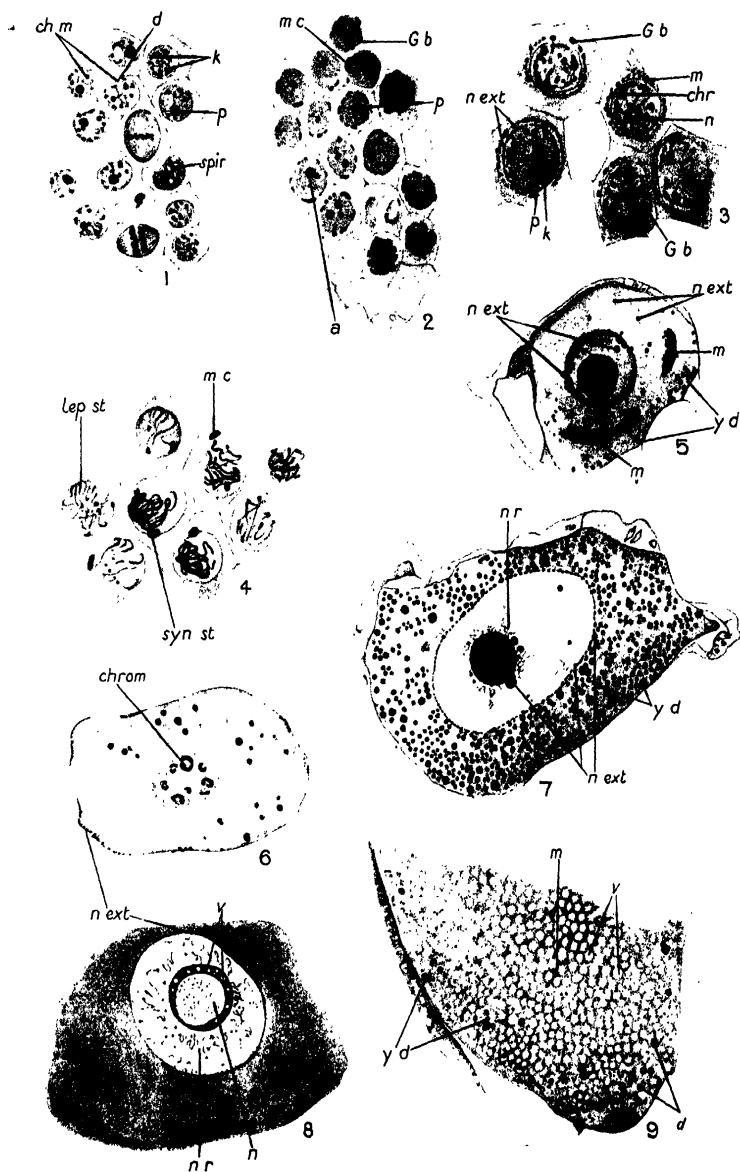
Fig. 14.—Oocyte about to undergo maturation showing vacuolation in rim of nucleolus, circular chromosomes, and Golgi apparatus in diffuse condition. (Da F., E.H.).

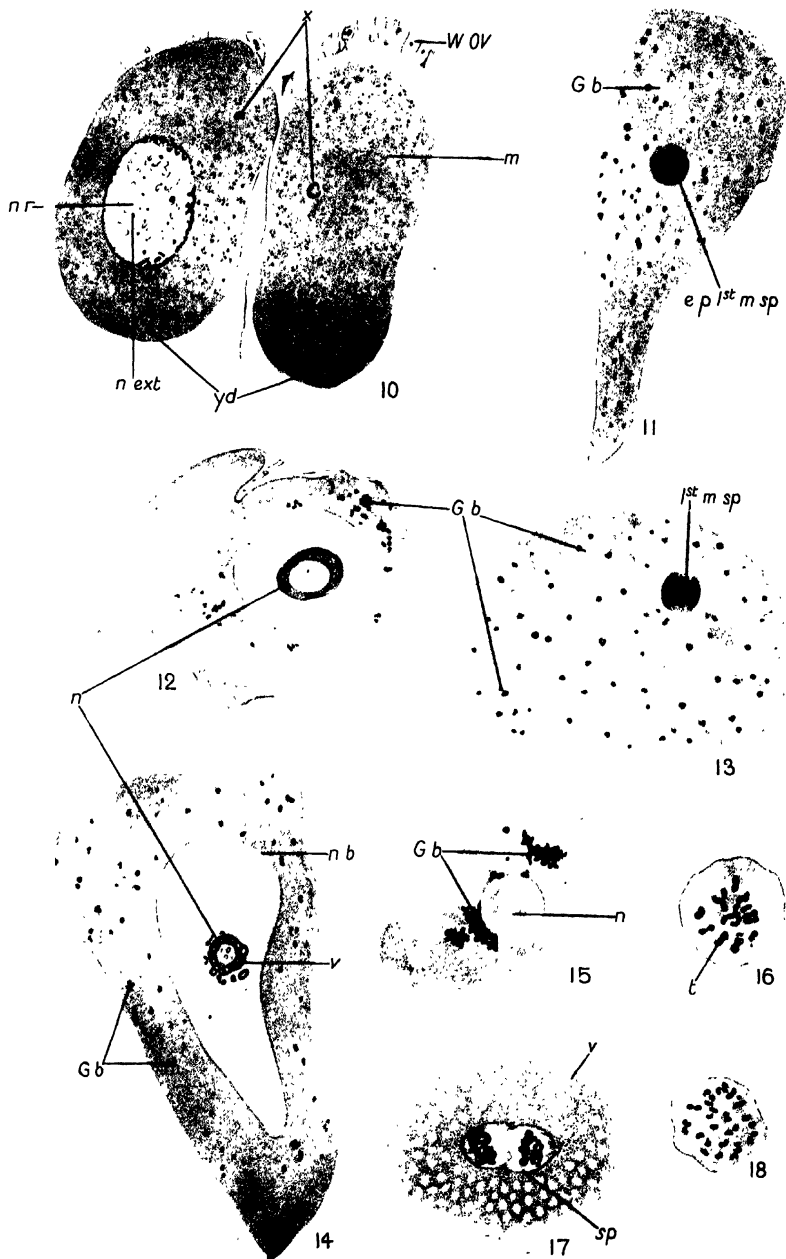
Fig. 15.—Young oocyte showing Golgi apparatus in complex condition. (Da F., E.H.).

Fig. 16.—Equatorial plate of first maturation division showing tetrads (magnification 1000 diameters). (F., I.H.).

Fig. 17.—Telophase of second maturation division. (F.w.a., I.H.).

Fig. 18.—Equatorial plate of second maturation division (magnification 1000 diameters). (F.w.a., I.H.).





The Structure of the Clitellum of *Alma emini*, Mich.

by

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With 4 Text-figures.

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INTRODUCTION.

THROUGH the kindness of Dr. J. Stephenson, I have been able to examine a specimen of *Alma emini*, Mich. (from Tanganyika), a Glossoscolecoid belonging to the sub-family Microchaetinae. This worm possesses several remarkable features, particularly, an unusually long clitellum, the investigation of which seemed likely to throw some light upon the relation of the clitellum to the generative apertures in the evolution of the Oligochaeta, tentative suggestions regarding which were put forward by me as a result of the observations upon the reproductive processes of the Lumbricids *Lumbricus* and *Eisenia* (Grove, 1927, p. 471).

In the Glossoscolecidae, the more usual position for the clitellum is in the region of the male pores, a condition which obtains in the sub-families Glossoscolecinae (except *Opisthodrilus*), Sparganophilinae and Hormogastrinae. In the Microchaetinae, however, while in the genera *Microchaetus*, *Tritogenia*, *Glyphidrilus* and *Callidrilus* the clitellum is in the region of the male pores, it lies well behind these apertures in the genera *Kynotus*, *Drilocrius*, and

Alma. This latter condition is also found in the Criodrilinae with its solitary genus *Criodrilus*. A distinctive feature of many of the species of these four genera is the unusual length of the clitellum (e.g. *K. kelleri*, twenty-six segments; *D. bürgeri*, thirty segments; *A. pooliana*, sixty-one segments; *C. lacuum*, thirty-two segments), but in the genus *Alma*, in addition to the often extreme length of the clitellum, the worms are characterized by the development, in connexion with the apertures of the vasa deferentia, of long processes to which is ascribed the function of the transference of the spermatozoa during coition. The position of the spermathecae, too, is remarkable; for, instead of occupying the more usual position anterior to the male pores, they are found either within or immediately in front of the clitellum.

In the Lumbricidae, the investigations upon the reproductive processes showed that the clitellum participated in both coition and cocoon formation and that the posterior position of the clitellum had associated with it a conveying mechanism by which the spermatozoa during the former, and the eggs during the latter, were conveyed from the generative apertures to the clitellum. In *A. emini*, however, the posterior position of the clitellum is combined with the development of a special mechanism in the form of penial processes for the conveyance of the spermatozoa during coition, and the clitellum consequently does not participate actively in the process, a condition which should result in corresponding differences in structure.

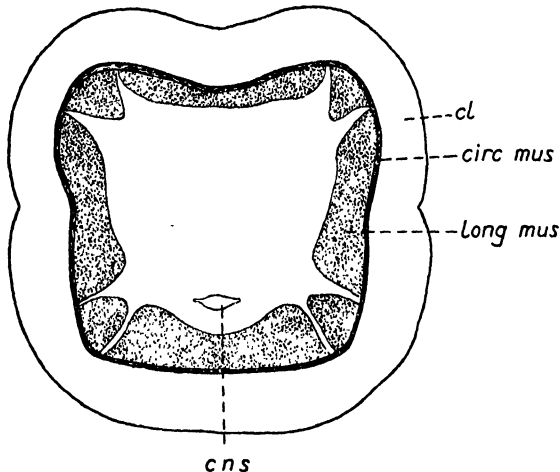
THE CLITELLUM OF *ALMA EMINI*.

The specimen of *A. emini* examined is intermediate between the two hitherto distinguished forms (Stephenson, 1928) the position of the clitellum agreeing almost exactly with that of the var. *Aloysii-Sabaudiae*, whilst the number and arrangement of the spermathecae conform more closely to that of the type. The clitellum is not clearly demarcated from the other segments of the body apart from a slight difference in colour. It extends from segment 55–100 (forty-six segments) and lies far behind the male pores, which are situated on penial processes arising from segments 18–20. The shape of the body behind the

penial processes is markedly quadrilateral and this persists throughout the clitellar region. No papillae are visible on the clitellum.

In a transverse section it is seen that, in spite of its lack of prominence externally, the clitellum is well developed, its glandular elements comprising more than one half of the thickness of the body wall. Its shape is roughly quadrilateral, but along the

TEXT-FIG. 1.

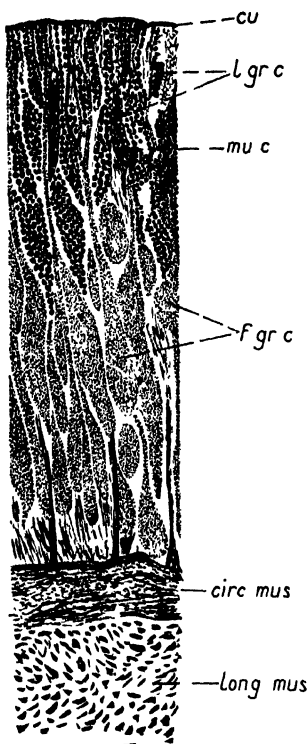


Semi-diagrammatic section through the clitellum of *A. emini*.
circ mus, circular muscles; *cl*, the thickness of the clitellum;
cns, central nervous system; *long mus*, longitudinal muscles.

sides a groove is evident, accentuated doubtless by the preserving process, and the ventral surface is more convex than the dorsal. Duboscq (1902) describing the clitellum of *Alma Zebangii*, states: 'Dans les anneaux du clitellum, le tegument tout entier, dorsal et ventral, a subi la transformation glandulaire, laquelle accentue les sillons normaux au lieu de les effacer.' Much the same conditions were found in *A. emini*, for the distribution of the glandular elements is uniform throughout the circumference, and there is no obvious difference in the structure of the clitellum such as was found in *Lumbricus* and *Eisenia*, dividing it into regions. Although the material

had been merely preserved in alcohol without the use of special fixatives, it was possible to differentiate the various glandular

TEXT-FIG. 2.



A portion of the clitellum of *A. emini* ($\times 266$). *cu*, cuticle; *circ mus*, circular muscles; *f gr c*, gland cells with fine granular contents; *l gr c*, gland cells with large granular contents; *long mus*, longitudinal muscles; *mu c*, mucin-secreting cells.

elements by the use of muci-haematin and picro-indigo-carmin, and also Mallory's triple-connective tissue stain (Grove and Cowley, 1927). In these preparations the same three types of unicellular glands were distinguished as were found in *Lumbricus* and *Eisenia*, viz., (a) mucin-secreting gland-

cells, (b) gland-cells with large granular contents, and (c) gland-cells with fine granular contents. The mucin gland-cells are fairly numerous, distributed rather irregularly, and somewhat elongated in shape, penetrating to some distance into the thickness of the clitellum. The large-granule cells are very numerous, uniformly distributed, and they too are elongated in shape extending still deeper into the thickness of the clitellum than the mucin gland-cells. The fine-granule gland-cells are arranged in groups separated from one another by sheets of connective tissue, the cells in each group forming columns in precisely the same way as was found in *Lumbricus* and *Eisenia*. Dubosq (1902) does not distinguish the mucin-secreting cells from the other superficial cells, but describes the large-granule cells, and the fine-granule cells he includes in a third layer as 'une couche de longues cellules muqueuses qui forment les trois quarts de l'épaisseur du clitellum'.

Apart from these characteristic constituents of the clitellum, no other glandular elements were distinguished such as were present in the Lumbricidae associated with the tubercula pubertatis or diverticula of the setal pores.

In view of the rather specialized character of *A. emini*, it was thought desirable to compare the structure of its clitellum with that of a more typical Glossoscolecoid, and, again through the kindness of Dr. Stephenson similar sections of the clitellum of *Diachaeta exul*, Steph., belonging to the Glossoscolecinae, were prepared.

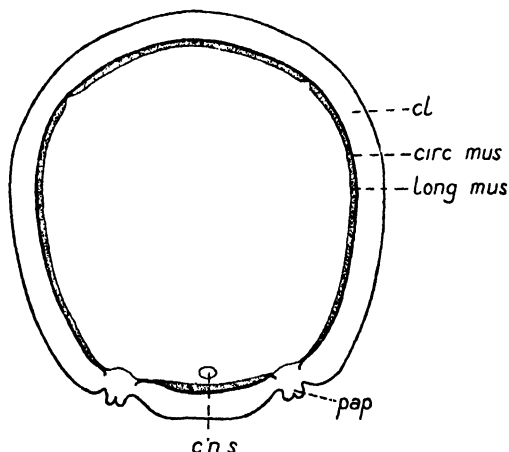
THE CLITELLUM OF DIACHAETA EXUL.

The clitellum in this worm is only seven segments long, occupying segments 14-20. In the spirit specimens it is clearly marked in an external view by both prominence and colour, its smooth greyish-white surface contrasting with the darker colour of the other segments. The segments in the clitellum are clearly defined, and on the ventral surface of the eighteenth segment are found the male pores. These lie at the posterior ends of crescentic papilla-like structures which extend forwards across the anterior half of segment eighteen and invade the posterior border of the seventeenth segment. Apart from the setae,

the only other structures which are obvious externally are a series of pit-like depressions on the lateral sides, in line with the lateral setae and situated in the furrows between segments 14/15 to 20/21, with indications of smaller depressions in furrows 13/14 and 21/22.

In a transverse section through the middle (segment eighteen) of the clitellum, the outline is roughly circular with no marked

TEXT-FIG. 3.



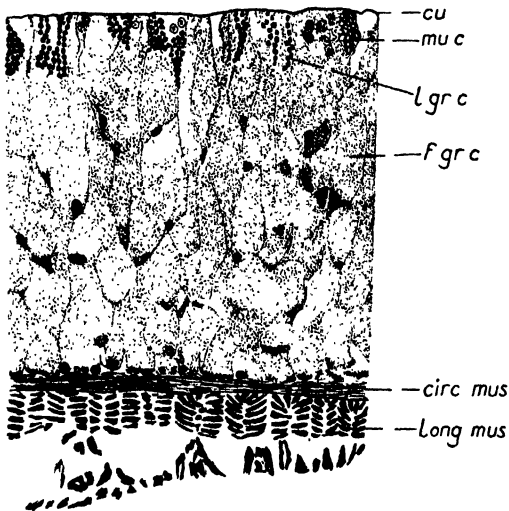
Semi-diagrammatic section through the clitellum of *Diachaeta exul*. *circ mus*, circular muscles; *cl*, the thickness of the clitellum; *c'n s*, central nervous system; *long mus*, longitudinal muscles; *pap*, papilla.

divisions into regions in accordance with the distribution of the glandular elements. The glandular portion comprises the greater part (3/4ths) of the thickness of the body-wall, and is uniform in thickness around the whole circumference, there being no appreciable thinning on the ventral surface. The same three types of unicellular glands are distinguishable as in *Alma*. The mucin-secreting glands are somewhat irregularly distributed and are relatively small in size, not penetrating deeply into the thickness of the clitellum. The large-granule containing cells too, although penetrating deeper than the mucin cells, are

relatively superficial. The fine-granule containing gland-cells have the same characteristic shape and distribution as in *Alma* except that the cells are much broader relatively to their length, and the narrow duct-like prolongations leading up to the surface are not so clearly defined.

The crescentic papilla-like structures found in relation with

TEXT-FIG. 4.



A portion of the clitellum of *D. exul* ($\times 266$). *circ mus*, circular muscles; *cu*, cuticle; *fgc*, gland cells with fine granular contents; *lgrc*, gland cells with large granular contents; *long mus*, longitudinal muscles; *muc*, mucin-secreting cells.

the apertures of the vasa deferentia contain none of the specialized clitellar glands. Scattered isolated mucin cells were found in preparations stained with muci-haematin and picro-indigo-carmin, but with Ehrlich's haematoxylin the papilla appeared to be covered externally by an epidermis of short cells with well-defined nuclei, and internally, to consist of a stroma composed of irregularly arranged cells with scattered nuclei. In transverse section, each papilla is composed of two prominent lips with a deep furrow between, and is in connexion with the mass

of cells which penetrate the layers of muscle of the body-wall, projecting into the body cavity. The vas deferens passes through this collection of cells and opens into the base of the furrow. It will be seen also, that these structures do not invade the glandular portion of the clitellum, and, apart from them, no other glandular structures are present.

The pit-like depressions that are so obvious on the external surface mark the apertures of the nephridia.

DISCUSSION.

It is evident that so far as its general glandular constitution is concerned, the clitellum of *A. emini* does not depart from that found in the more typical Glossoscolecoid *D. exul*. In addition, the gland-cells present show a marked similarity in their constitution and arrangement to those which, in the Lumbricidae, have been shown to be concerned with cocoon secretion. In *A. emini* we have an example of an Oligochaete in which the development of special penial processes in connexion with the apertures of the vasa deferentia has removed from the clitellum the necessity of active participation in coition such as has been associated with its posterior position in other cases. The position of its spermathecae, opening as they do in the furrows between segments 52/53-84/85, suggests that the spermatozoa will in all probability be deposited on the clitellum, but the clitellum itself will play a more passive role than where it is concerned, as in *Lumbricus* and *Eisenia*, with the collecting of the spermatozoa around the apertures of the spermathecae. (In this connexion it must be remembered that in other species of *Alma* the spermathecae may lie entirely in front of the clitellum—e.g. *A. stuhlmanni*, *A. schultzei*, &c.—and consequently the clitellum will not be included in the region to which the penial processes would be applied, so that even in *A. emini* it can be regarded as not participating as such in the process of coition.) The remaining outstanding feature is therefore its length.

No description of the cocoon of *A. emini* appears to exist, but that of *A. multisetosa* has been described by Michaelsen (1915, p. 304): 'Die Kokons sind ungefähr 130-155 mm. lang

und in der Mitte 9–10 mm. dick, langspindelförmig, an den Enden dünner werdend und schliesslich in sehr dünne, etwa 10–15 mm. lange Endschläuche ausgezogen.' *A. multisetosa* is a large worm (Michaelsen, 1915, p. 280) more than 240 mm. long and 8 mm. thick, the clitellum extending from 80–136 segments (fifty-seven segments). Dr. Michaelsen has very kindly lent me one of the four cocoons from which his description was made. This had been opened and found to contain thirty-two embryos in a fairly advanced stage of development. The cocoon membrane had much the same appearance as that of the cocoons of *Lumbricus* and *Eisenia*. The age of the cocoon, as evidenced by the advanced development of the contained embryos, rendered it extremely unlikely that any remains of a cocoon slime-tube would be found, and careful examination failed to reveal anything which could be determined as a slime-tube. Nevertheless, the presence of mucin secreting glands in the clitellum would provide the means for the production of this structure.

The only other Glossoscolecoid cocoons that I have been able to examine are those of *Criodrilus lacuum* in the collections of the British Museum (Natural History). In this worm the clitellum is also long (Michaelsen, 1900, gives it as more than thirty segments, and in a specimen examined, it appeared to extend from segment 16 to 47). The cocoon, too, is long, narrow, and spindle-shaped, with long-drawn-out prolongations at either end. These prolongations vary in different cocoons and it has been impossible to determine whether a slime-tube is present or not.

It is a remarkable occurrence that in both of these worms, each of which is distinguished by the possession of an extremely long clitellum, the cocoon has this unusual elongated-spindle shape so unlike that of most Oligochaete cocoons. The obvious conclusion that may be drawn is that these two conditions are intimately associated. Michaelsen (1929) discussing the question of the extent of the clitellum in the Oligochaeta states that the length of the cocoon is determined by the size and number of worms which complete development, thus (p. 697): 'Schon bei den relativ kleinsten Kokons muss die Gürtellänge die Dicke des Wurmes beträchtlich übertreffen. Hat der Kokon mehrere

und relativ grössere Jungwürmer zu liefern, muss also sein Inhalt grösser sein, so kann er dies nur durch Zunahme seiner Länge erreichen, denn die Kokondicke, entsprechend der Dicke des Wurmes, ist beschränkt.'

As already noted above, the long cocoon of *A. multisetosa* contained thirty-two embryos, but there is no evidence as to what proportion of the original number of eggs laid this represents or the number which will eventually reach maturity. Orley (1887) records that in *Criodrilus* the number of eggs varies from 8-20, but that usually only about one-third of the number develop, the largest number of embryos found being eight and the smallest two.

It is possible, though its significance is not easy to see, that the spindle-shaped cocoon produced by these two worms may have some connexion with their particular habitat, for in both cases the cocoons are produced at the time the muddy swamps in which they live are drying up.

So far as *A. emini* is concerned, therefore, all that can be said with certainty is that the extreme length of the clitellum is merely a concomitant of the unusual length of the cocoon, and has no relation to the other reproductive processes. It is of interest that the glandular constituents of the clitellum support the view that it is concerned with cocoon formation only, as would be expected from the development of the special penial processes, but this interest is enhanced by conditions found in *D. exul*, for in that worm also the glandular constituents of the clitellum proper are solely those which in the Lumbricids are concerned with cocoon formation. This, in view of the close association of the male apertures with the clitellum is rather unexpected and further work on the process of coition in the Glossoscolecids, which, however, would only be of value if carried out with living material, is necessary before any definite statement could be made. It is possible that the secretion of the glands in connexion with the crescentic papillae associated with the apertures of the vasa deferentia may suffice to effect any attachment necessary during the transference of the spermatozoa, or that the papillae themselves may operate in the same way as described by Bahl (1927) in *Eutyphoeus*.

Many points have come to light during the examination of sections prepared during this investigation which suggest the possibility of the existence of a carrying mechanism for the backward conveyance of the eggs into the cocoon prior to deposition; and the presence of the spermathecae in the clitellar region offers a simple explanation of the entry of the spermatozoa. But the absence of opportunity for observing these processes in the living worm precludes the possibility of elaborating these further at present.

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The Oogenesis of Lumbricus : a Restatement.

by

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With Plate 11 and 5 Text-figures.

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INTRODUCTION.

DURING the last seven years immense progress has been made in the study of the form and function of the Golgi apparatus in animal cells, and there are few cytologists who would deny that the secretion granules of gland-cells arise in intimate relation to the Golgi apparatus. On the question of its relation to vitellogenesis, although a large bulk of observations has been recorded, there is a baffling diversity of results. So much so indeed that many workers have concluded that yolk arises in a diversity of ways, each egg being a law unto itself. In previous papers I have combated this theory, partly on observational grounds, and partly on the theoretical ground that the storage of yolk in eggs is so universal a phenomenon that it must be very primitive, and hence there is probably some basic method of formation to which all variants can eventually be referred.

Recently a certain amount of work has been published independently on which I have ventured to enlarge Hogben's theory of yolk-formation (Hogben, 1921). This theory states that yolk is formed in eggs by the interaction of plasmosome, mitochondria, and Golgi apparatus. I have further suggested (1929) that the

mitochondria are concerned in yolk synthesis from raw material derived from plasmosome, ground cytoplasm, and from sources external to the egg, sometimes via nurse-, follicle-, or test-cells, and that the function of the Golgi apparatus is the isolation of the material so formed out of the cytoplasmic solution.

Obstacles to this theory are offered by a series of papers on eggs by Nath and his collaborators ('Studies on the Origin of Yolk', 1928, 1928, 1929, 1929, 1930; 'Studies on the shape of the Golgi Apparatus', 1929, 1930). The evidence offered by these workers is to the effect that (1) the Golgi apparatus occurs in the form of vacuoles, (2) in the majority of the eggs studied fat becomes deposited in these vacuoles, which enlarge and become fatty yolk, and (3) proteid yolk arises from different sources in different eggs. These results have puzzled me exceedingly, and it was only on the publication of Nath's recent paper on the ovary of *Pheretima* (Nath, 1930) that any way out of the impasse appeared. In this paper Nath speaks in general on the oogenesis of earthworms, embracing the results of Gatenby and Nath (1926) on *Lumbricus*, and states that there is no yolk in the eggs, that the Golgi bodies are vacuolar and slightly fatty; findings quite contrary to my own (1925) on *Lumbricus*. Since therefore this egg may hold the key to our differences on the wider question of yolk-formation in general I may be excused for returning to my former work and revising and restating my views.

The work has been carried out in the Department of Zoology of the University of Edinburgh. I am indebted to Mr. R. J. Fant of this Department for the excellent photographs of living eggs.

MATERIAL AND METHODS.¹

The species of worm used was *Lumbricus terrestris*. The animals, captured overnight, were opened and the ovaries removed and examined in a fluid consisting of 1 part sea-water in 4½ parts of distilled water. This fluid was suggested to me by Mr. A. D. Hobson, M.A., of this Department. It is an excellent physiological solution for the eggs of *Lumbricus*, causing no

¹ Expenses incurred in this research have been defrayed by a grant from the Earl of Moray Endowment of the University of Edinburgh.

swelling or plasmolysis, the eggs remaining perfectly healthy for long periods. The method yielding the best results was that of examining fresh unstained ovaries. Staining in neutral red and brilliant cresyl blue was also very useful. I have not found Nath's technique of fixing lightly in weak osmic acid very helpful and I cannot confirm his statement that it renders the Golgi bodies more visible. On the contrary I find that the refractive index of the cytoplasm is raised relatively to that of the Golgi bodies, and they are then far less easily seen. The fat droplets become markedly more prominent and develop a pseudo-rim of darker hue owing to their high refractive index, and to a darkening of their contents in the osmic acid solutions (*vide infra*).

As tests for fat I have used Nile blue sulphate, Sudan III, and Scharlach R on both fresh and formalin-fixed material. Ciaccio's method for unmasking lipoids (see Bowen, 1928) was used with success on the Golgi apparatus, the ovaries being mounted whole in glycerine jelly.

All results have been checked on material fixed by routine methods.

The use of dark-ground illumination has not proved so successful on this ovary as I had hoped. The mitochondria are so small and crowded that a general milky effect is produced in which it is difficult to see individual elements, and the Golgi bodies are quite invisible except when lying in an area thinly strewn with mitochondria. In the latter event they show as optically empty spaces in the glistening milky area. This is not the case in all eggs, although I have found none in which dark-ground work facilitates the study of the Golgi apparatus.

The photography was carried out with a Zeiss FZE stand using an apochromatic 2 mm. objective and a 7 compensating ocular. For dark-ground photographs a cardioid condenser with special 3 mm. objective was used. The source of light was a Pointolite 150 cp. bulb. A short camera extension was found best, about 8 inches being used in most cases. The exposures were made on Wellington anti-screen plates of speed 450 H & D.

LITERATURE.

There is no need further to review the literature on annelid oogenesis. This may be referred to in the recent papers by Harvey (1925), Gatenby and Nath (1926), Nath (1930), and Weiner (1930), a particularly full list of references being given in the latter paper. Nath's views (1930) are that there is neither proteid nor fatty yolk in the egg, and that the Golgi bodies are 'refractile spherules of dark-greyish colour', containing a small percentage of free fat. The mitochondria are granular. Vital stains (neutral red and Janus green B) have little effect on the cell and do not stain the Golgi bodies at all. The importance of treating fresh ovaries with weak osmic acid solution is emphasized, the result obtained being a slight blackening of the Golgi bodies after a half hour, and their differentiation into lighter interior and darker rim, thus demonstrating their vesicular nature. O'Brien and Gatenby (1930) have recently expressed agreement with these views, except that they used neutral red with success.

At about the same time as Nath's paper on *Pheretima* there appeared a paper by Weiner (1930) on *Allolobophora calliginosa* and *Eisenia rosea*. This author found quite a different state of affairs. He confirms my original statement (1925) that the mitochondria are filamentous as well as granular, and also that there is some nutritive material stored in the cytoplasm. This material is however not yolk as I had suggested, but a very weak fat. The Golgi bodies seen by Weiner are not vesicular, but scale-like, and Ciaccio's method for lipoids was found to give a positive result on these scales. Neutral red was used 'vitaly' and stained minute vacuoles lying at first on the concave sides of Golgi bodies, and later moving away from them. The fatty droplets arise in the cytoplasm showing no positional relationship to any of the formed elements.

My own observations (1925) were that the mitochondria are granular and filamentous, arising as a cap over the nucleus and spreading out gradually until the elements are widely scattered. I could not then find definite mitochondrial elements in the oogonia and early oocytes, but later observations have con-

vinced me that they are present. The Golgi elements I considered as probably platelets or spheroids, not rodlets, and I was not, and still am not, convinced that they arise from a single Golgi body in the oogonium. I also observed droplets present in the eggs from a very early stage, even in the oogonia, and these for want of a better name I called yolk-droplets. They arose *de novo* in the cytoplasm. Calkin's yolk-plates were also present in many of my preparations but I was unable to discover any evidence as to their origin and function.

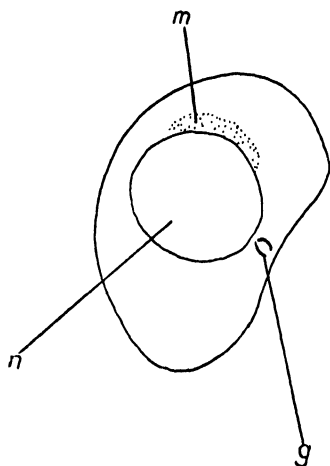
OBSERVATIONS.

On the history of the constituents of the egg other than the Golgi apparatus and yolk, I have very little to add to my earlier observations (1925). I have however satisfied myself that the mitochondria are present as a cap on one side of the nucleus in the earliest oogonia.

The Golgi apparatus of the living oocyte has been studied most closely in the eggs towards the tag end of the ovary. Contrary to the statements of Gatenby and Nath (*loc. cit.*) I have found that the younger oocytes are difficult to study living, or freshly killed in osmic acid. They are small, with very little cytoplasm, and extremely closely packed. In addition the overlying membranes of the ovary are so thick and muscular that they render critical observation of single oocytes almost impossible, and I should hesitate to dogmatize on this ground alone as to the number of Golgi bodies present in each oocyte or oogonium. Consequently my observations on living cells *in situ* refer chiefly to the older oocytes. Here the mitochondria are spread in large, irregular clumps of granules and filaments all over the cytoplasm, in active Brownian movement. The Golgi bodies are visible under critical illumination as short, fairly stout rodlets usually slightly curved in crescent form (Text-fig. 4; figs. 1, 2, and 3; Pl. 11). They are especially prominent in the neighbourhood of the nucleus and often are more concentrated at one side than elsewhere. There are Golgi bodies scattered also through the entire cytoplasm, but here they are fewer and less prominent because of the greater proportion of mitochondria and fat droplets which tend to obscure them.

All that can be seen of the living Golgi body is a short curved rodlet, tapering at each end and of slightly higher refractive index than the cytoplasm. Frequently two or three of them are seen closely apposed, as if encircling a central sphere, but the material of this central area does not seem to differ optically from that of the ground cytoplasm of the cell. At certain foci it appears as a lighter patch, and a similar lighter area can be

TEXT-FIG. 1.



TEXT-FIG. 2.

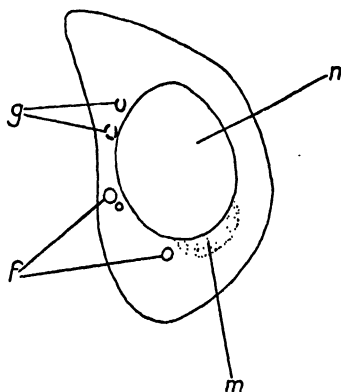


FIG. 1. Diagram of a living young oocyte. *n*, nucleus; *m*, mitochondria; *g*, Golgi body. Mag. 700.

FIG. 2. Young oocyte of about the same age as Text-fig. 1, with three fat droplets and two complex Golgi bodies. *f*, fat droplet. Other lettering as in Text-fig. 1. Mag. 700.

seen at times alongside the single rodlets, but this is an optical effect due entirely to the refractive property of the rodlet and not to any differences in the cytoplasmic matrix. That there is material of special properties, 'sphere substance', associated with the Golgi bodies can only be demonstrated after fixing and staining with iron haematoxylin, when the Golgi elements appear as dark blue rodlets each with a patch of grey material on the concave side (Text-fig. 5). This appearance can be obtained after any of the recognized chrome-osmium fixatives (Champy,

Flemming-without-acetic, &c), and also after osmic impregnation methods, when the rodlet appears black. After Ciaccio's method for unmasking lipoids the Golgi bodies are present in cells of all ages as brilliant orange-red rodlets. The sphere substance does not appear after this technique. Whether this patch of material exists in two dimensions or in three it is difficult to determine. I am inclined to think it has three, but in any case there seems to be no limiting membrane, a conclusion which is supported by the fact of its invisibility in living cells. It is quite certain however that the Golgi bodies are not spheres with the rim differentiated from the internal substance. The only spheres seen in the egg and at all comparable in size with the Golgi bodies of fixed oocytes are the fat droplets, and these show no relation to the Golgi apparatus.

It is possible to study the younger oocytes by pressing the ovary between coverslip and slide until the membranes are broken, when the germ-cells are squeezed out and can be examined with comparative ease. In the majority of these younger cells the Golgi apparatus is obscured by the mitochondria, which lie as a close cap over one side of the nucleus. Apart from this mitochondrial cap the only constituents which can be seen in most of the oocytes are one or two fat droplets. Under favourable circumstances, however, two or three complex elements of the Golgi apparatus can be seen, each consisting of several rodlets like those occasionally seen in older oocytes (Text-figs. 1 and 2). Occasionally single rodlets can be seen. I must confess I have found it extremely difficult to be certain of the Golgi apparatus in the very earliest oocytes and the oogonia. The cytoplasm forms so thin a film over the nucleus that frequently it is difficult to see more than a small mitochondrial cap. Among the mitochondria can be made out sometimes one and sometimes more Golgi bodies, but after spending some days observing these cells I am still unconvinced that there is only one element present in the oogonium from which all those of the oocyte evolve. In many cases it appears, as claimed by Gatenby and Nath (1926), Nath (1930), and Weiner (1930) that there is only one element present, but in equal numbers of cells no more advanced than these at least two Golgi bodies are

present and sometimes more. Taking into account therefore the difficulty of being certain in vital observation that the whole of a cell has been examined I am inclined to leave the matter open.

It is suggestive that there is a much higher percentage of complex Golgi bodies in the growing oocytes than in those fully formed. The obvious inference is that they represent a phase in the multiplication of the apparatus by division, and hence that the Golgi bodies arise by division of pre-existing similar elements.

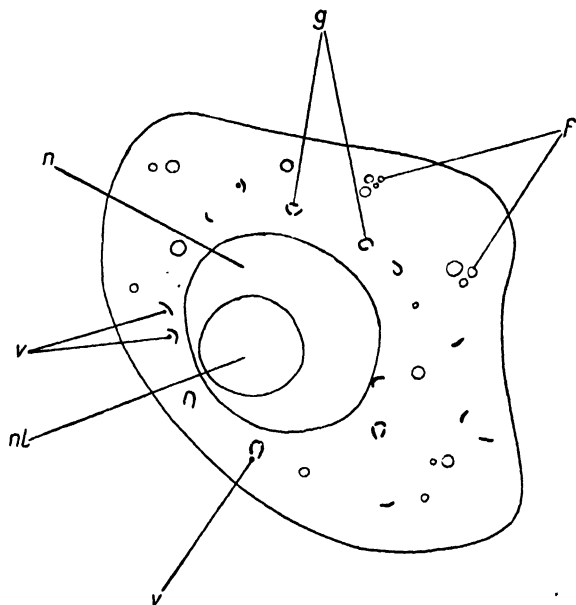
The difficulties of observation of the younger oocytes are magnified enormously under dark-ground illumination, and in consequence such work has been confined to the older oocytes. Under this lighting system the Golgi bodies are not usually seen at all. Only those elements lying in an area thinly strewn with mitochondria can be seen, and then merely as negative pictures. The mitochondria cause a general milky scintillation of the cytoplasm, and against this light ground the Golgi body can be seen as a black rodlet. In many instances an oocyte was examined under direct illumination, and the Golgi bodies were seen in comparatively large numbers at one side of the nucleus. On subjecting the same cell to dark-ground illumination (an operation occupying not more than a minute) the area at the side of the nucleus occupied by the Golgi bodies appears optically empty. Reversion to direct light shows the cell to be quite unchanged.

Treatment of the oocyte with osmic acid for periods from 10 minutes to 24 hours and examination in water yielded very little. The refractive index of the cytoplasm is raised relative to that of the Golgi bodies and the latter deepen very little in colour even after 24 hours. Hence many of the Golgi bodies can no longer be seen, and those that remain visible gain nothing in clarity, appearing as thin brownish curved rodlets in a brown cytoplasm. If ovaries so treated are mounted in balsam after dehydration and clearing in xylol, the rodlets can still be seen.

No positive effect on the Golgi bodies was observed after staining with neutral red or brilliant cresyl blue, nor after Scharlach R and Sudan III on living or formalin-fixed ovaries. The use however of a strong solution of Nile blue sulphate on

fresh ovaries frequently enables the Golgi rodlets to be stained quite sharply blue. The sphere substance does not stain. Further, staining with Scharlach R after digestion of fixed ovaries in 1 per cent. phenol at 37° C. for 24 hours demonstrates the lipoidal nature of the rodlets, which are coloured bright

TEXT-FIG. 3.



Half-grown living oocyte at an early phase of neutral red staining.
nl, nucleolus, *v*, neutral red vacuole. Other lettering as in previous figures. Mag. 700.

orange red. The fact that the sphere substance does not stain by this method suggests that the lipoidal material of the Golgi element is confined to the rodlet.

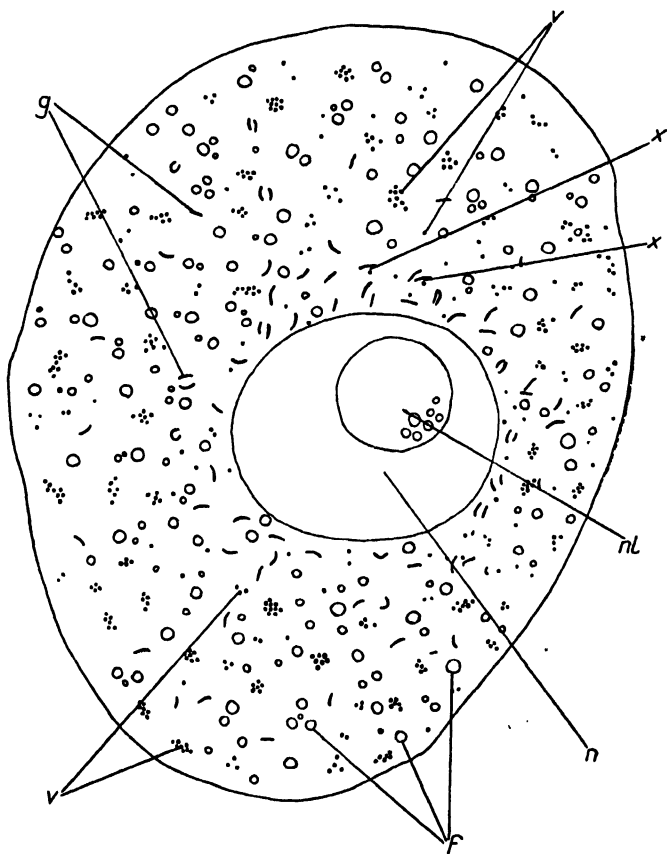
Besides Golgi bodies and mitochondria there can be seen in the living oocytes refractile colourless spherules, varying in size from the minute to a diameter of about 2 micra. In some ovaries they are not present in the oogonia and young oocytes, but in others one or two droplets may be seen even in the oogonia,

often embedded in the mitochondrial cap, or lying close to it. In the fully grown oocyte they are sufficiently numerous to be a prominent feature, lying scattered over the entire cytoplasm but being fewer in the immediate neighbourhood of the nucleus (*f*, Text-fig. 4; figs. 1-4, Pl. 11). Treatment of the ovary with weak osmic acid causes these droplets to darken in colour. After half an hour they are distinctly browner than the ground cytoplasm, and owing to their increased refractive index relative to the cytoplasm they have developed an apparent rim. After 24 hours in osmic acid the spherules are dark brown and the apparent rim is still more prominent. There is no true rim differentiated at any time however, the effect being entirely optical, occurring at certain foci only, and it is not visible when the equator of the sphere is in focus. After examination in water some of the osmicated ovaries were brought up through alcohols and xylol and mounted in balsam. It was found that the contents of the spherules were dissolved out on reaching xylol, leaving empty vacuoles in the mounted oocytes. Treatment of the ovary with Nile blue sulphate stains the spherules very pale blue. This blue is so weak that the result may be regarded as negative. After Sudan III, however, they are pale yellow, and after Scharlach R deep scarlet. Under dark-ground illumination the droplets form by far the most prominent feature of the cell, being brightly outlined, and showing quite marked Brownian movement. They resist solution in alcohol up to 70 per cent. strength, but are soluble in 90 per cent. As a result of these observations it can be safely concluded that the spherules are fatty in nature. The result with Nile blue sulphate indicates that the solution is comparatively weak, thus confirming Weiner's observation (1930).

In the eggs of ovaries placed in 1/20,000 neutral red solution in dilute sea-water for from 80 to 90 minutes, the vacuome can be seen. This is in the form of extremely minute bright orange-red vacuoles of low refractive index. In the early stages of staining they show a tendency to be more concentrated in the immediate perinuclear area, but in well-stained eggs they are scattered throughout the cytoplasm in little clumps of half a dozen. In the early stages of staining it is seen that many of

the vacuoles are lying on the concave sides of the Golgi rodlets or enveloped by two or three elements, and the first vacuoles

TEXT-FIG. 4.



Full-grown oocyte at a late stage of neutral red staining. Lettering as in previous figures. At *x* note neutral red vacuoles still in association with Golgi bodies. Mag. 700.

to arise in the egg always occupy this position (Text-fig. 3). Later, however, when the little scattered clumps of vacuoles are formed, very few of the Golgi bodies have a vacuole associated

with them (Text-fig. 4). It is clear then that the vacuoles arise in connexion with the Golgi apparatus but later wander away from it into the cytoplasm. Text-fig. 3 shows a slightly younger oocyte in which staining has not proceeded very far. There are only two or three vacuoles formed and these all lie on the concave sides of Golgi rodlets.

In the younger oocytes there are usually from ten to twenty of the vacuoles present, scattered over and among the mitochondria of the cap. No vacuoles were seen in the youngest oocytes or in the oogonia. This does not necessarily mean that they cannot be found, but simply that the technique has failed to demonstrate them. The difficulties of staining the oocytes increase as one progresses towards the younger end of the ovary.

Owing to the fact that the vacuoles are very similar in size to the mitochondria it is difficult to be certain that they are not present in the cell before the application of the stain. Very few Golgi bodies however can be seen in unstained oocytes with a vacuole or granule of any sort on the concave side, and this, coupled with the fact of their migration away from the Golgi bodies in the later phases of staining, and their accumulation in clumps, renders it highly probable that they are secondary bodies formed as a reaction of the cell to neutral red, and that they do not exist in normal oocytes.

DISCUSSION.

I do not intend to discuss the question of yolk-formation in general on the grounds of these observations on the eggs of *Lumbricus*. This will be reserved for a later paper which it is hoped will shortly be ready for publication.

I am now prepared to admit that, as Gatenby and Nath (1926) have claimed, there is no true yolk in the eggs of earthworms. The material which, for want of a better word, I had designated as yolk proves to be a very weak fat, as shown by Weiner (1930). It has been difficult to account for the statement of Gatenby and Nath (loc. cit.), reiterated by Nath (1930), that there are no elements present in the eggs of *Lumbricus* other than the mitochondria and Golgi bodies. I had considered the possibility of insufficient identification of species used, until

Nath made a sweeping generalization to the same effect embracing the eggs of all earthworms. The photographs of living eggs are I think sufficient evidence that this statement is untrue. There are at least three constituents of all full-grown oocytes of *Lumbricus*, the mitochondria, the Golgi rodlets, and prominent vacuoles of weak fatty nature. Further, these three elements can be found frequently even in the oogonia, although in some ovaries the fat droplets are absent except in the later oocytes.

Nath's position (1930) with regard to the oogenesis of earthworms may be summarized briefly as follows. The Golgi bodies are dark grey refractive spherules, formed by division of a single spherule in the earliest oogonia and repeated divisions of the daughter Golgi bodies so formed. The mitochondria are not visible in the earliest oogonia but soon appear as a cap of whitish, less refractive granules over the nucleus. They gradually spread out in the cytoplasm and perform a dancing movement. There are no other inclusions present in the cell. He has examined the eggs of both *Lumbricus* and *Pheretima* and found that they are to all intents and purposes identical in structure. Now comparison of Nath's figures of the oocytes of *Pheretima* with my photographs of *Lumbricus* eggs leaves little room for doubt that Nath has not seen the Golgi bodies of his animal at all, at least in the living oocytes. What he has called Golgi bodies are undoubtedly the fatty spherules which seem to be present in the eggs of all earthworms so far examined (vide Harvey, 1925; Weiner, 1930; Nath, 1930). The reactions of the spherules of *Pheretima* are similar to those of *Lumbricus* here recorded, and so also is their distribution in the cell. Nath himself has admitted that there is a small percentage of fat in his spherules.

Nath refers extensively to the excellent work of Foot (1894, 1896, 1898) and Foot and Strobell (1898, 1900, 1901) on the ovary of *Allolobophora foetida*. He confidently states that the 'osmophile granules' of these workers are the Golgi bodies, and the 'yolk-nucleus' or 'archoplasmic granules' are the mitochondria. The latter homology I do not dispute, but it is quite plain from their photographs, figures, and descriptions that the osmophile granules are simply the fatty granules

demonstrated by Weiner and myself, as Foot and Strobell were inclined to think. They did not see the Golgi bodies, and these are not figured in any of their illustrations.

The extraordinary confusion which has arisen over the structure of the earthworm egg seems to be due to two allied sets of facts: 1. The Golgi bodies in the living egg are not striking objects, although they can be seen easily under exactly critical illumination, whereas the fatty spherules are prominent. 2. In the fixed cell the Golgi bodies are easily seen both after silver and osmic impregnation methods and in chromeosmium and iron haematoxylin preparations as ring- or plate-like bodies of dual structure (*vide supra*), whereas the fatty spherules are difficult to preserve other than as minute colourless vesicles. Hence the Golgi bodies tend to be overlooked in living cells, and the fatty spherules in sections. Now the numbers of each of these elements in the mature oocyte are very similar and their distributions do not differ greatly, and hence they have been confused together. It will be seen, however, that there is a distinct concentration of Golgi bodies around the nucleus, although they are also scattered throughout the cytoplasm. The fatty spherules on the other hand are rarer in the perinuclear area than elsewhere in the cell.

The implications of this comparatively simple solution are far-reaching. Nath, in a series of papers ('Studies on the Origin of Yolk', 1928, 1928, 1929, 1929, 1930; 'Studies on the shape of the Golgi Apparatus', 1929, 1930), has claimed that the Golgi bodies of the eggs he has examined are vacuolar, and that in many cases at any rate fat is deposited in these vacuoles, which enlarge, and with few exceptions become fatty yolk. In none of the eggs which I have myself examined have I been able to convince myself of the vacuolar nature of the Golgi elements,¹ and in all those in which they can be seen in the living condition

¹ The criticisms of silver impregnation techniques which I have made after examination of living *Lumbricus* eggs (*vide infra*), apply with equal force to those of Ciona, in which I have described a spherular form of Golgi body. I have not been able to see the Golgi apparatus in living eggs of this Tunicate, but I feel certain that the osmic techniques give a much more accurate picture of the Golgi bodies than do silver methods.

the visible part of the element is a rodlet or scale. There is an invisible component in each case, which appears on fixation, but which never shows any limiting membrane. Moreover, in no egg have I found fat appearing in connexion with the Golgi apparatus, or with any other constituent of the cytoplasm. My observations on fat formation in eggs are consistent with the recent results of other workers (Weiner, 1925, 1930; Steopoe, 1926, 1929; Hibbard, 1928). The possibility that fat might arise in some eggs in connexion with the Golgi apparatus and not in others has always appeared doubtful to me, on theoretical grounds. It is still further removed from the bounds of probability by the discovery that in one, at least, of Nath's cases he is not dealing with Golgi bodies at all, but with droplets already of fatty nature, and having no relation to the Golgi apparatus.

I am asked by Nath to consider three fundamental points:

1. That there is an essential difference between a fat droplet or globule, and a 'vesicle having a definite membrane containing fat'. That this difference may exist I cannot deny, but that it has any significance seems extremely doubtful in view of the fact that what Nath has called vesicles in *Pheretima*, Foot and Strobell (loc. cit.), Weiner (1930) and I have shown to be droplets in other worms. The distinction seems therefore to be one of terminology. In *Lumbricus* the rim of the vesicle is probably entirely an effect of refraction and has no actuality. Further the essential point under consideration is not the form of the droplet or vesicle, but the method by which fat appears in eggs, with which is inevitably linked the question whether there is a uniform method of fat formation applicable broadly to all eggs.

2. Nath's second point, that the Golgi bodies are fatty even when they do not swell to form yolk, is answered by the observation that in one case at least such 'Golgi bodies' are in actual fact fatty droplets pure and simple.

3. The third point made is that the fat droplets of *Carcinus* (Harvey, 1929) and *Discoglossus* (Hibbard, 1928) appear during oogenesis, whereas there is fat in the Golgi bodies of even primordial germ-cells in the forms examined by Nath and his fellow-workers. This point I would suggest lends further weight, if any is required, to the suspicion that Nath is dealing not with

Golgi bodies at all, but with fat droplets. I have found in *Lumbricus* that fat droplets are present even in oogonia, so that it is not unlikely that they may also be present in the primordial germ-cells of other animals. That they appear at a later stage in some animals than in others is not of much significance, it being known that the time of appearance of yolk in eggs does not depend entirely on the age of the egg, but is also related to the breeding season.

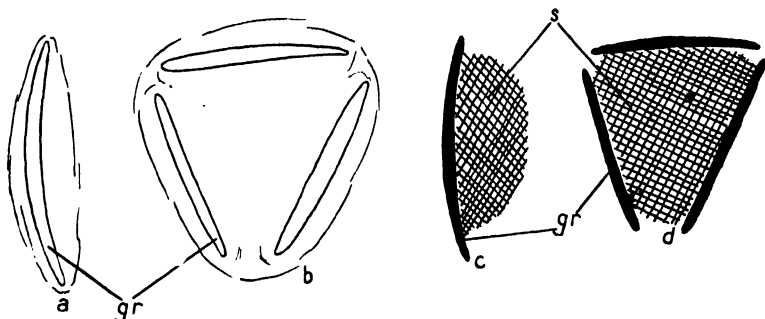
My earlier observations on the egg of *Lumbricus* (1925) led me to the conclusion that the Golgi bodies were in the form of small spheroids or platelets, something after the shape of a blood corpuscle. At that time I had not seen them in the living cell, and my deductions were made chiefly from material prepared by Da Fano's method, wherein the Golgi bodies are usually in the form of blackened rings, surrounding less impregnated central masses. I believed that this was the most complete picture obtainable of the Golgi apparatus of this egg, and that probably osmic techniques gave only a partial impregnation of the rim. Since examining the living eggs of *Lumbricus* and other animals I have been drawn to the conclusion that the osmic techniques give the more faithful picture of the Golgi bodies, and that silver techniques cause a greater or lesser amount of distortion and artefact, and in addition are far less specific in their action.

The Golgi bodies of the living oocytes of *Lumbricus* appear as refractive rodlets usually tapering towards each end and slightly curved (Text-fig. 5). Frequently two may be seen lying parallel with each other, in which case they are both almost straight. More rarely in older oocytes, but quite commonly in the younger ones, three or four shorter rodlets may be seen disposed as if on the perimeter of a roughly round body—the sphere material. Similar bodies are seen in the fixed and stained eggs, except that the rodlets are usually thinner and more sharply outlined than in the living condition, and the sphere material is now visible as a patch of grey material between the rodlets of the last two types mentioned, or at the concave side of single rodlets (Text-fig. 5). This type of Golgi body has been figured frequently in invertebrate germ-cells, particularly

in the male. In some instances it has been found to be concerned in the formation of granules—the acrosomal granule of male germ cells, and yolk-granules in some eggs—and the position of these on the surface of the sphere material proves that this is not a fixation artefact but has some counterpart in the living Golgi body. Further evidence to this effect is supplied by cells stained with neutral red *in vivo*, where the red vacuoles appear in positions similar to those occupied by the acrosomal and yolk granules.

In some cells the visible part of the Golgi body is a rodlet, in

TEXT-FIG. 5.



Diagrams to illustrate the relation between living and fixed Golgi bodies (osmic techniques). a. Single rodlet, living, showing shape and refractive phenomena associated with it. b. Complex Golgi body, living. c. Single rodlet fixed showing sphere material. d. Complex Golgi body fixed. *gr*, Golgi rodlet; *s*, sphere substance.

others it seems to be a scale, but the two forms are obviously mere variants of a fundamental structure. The Golgi bodies are therefore dual in nature, having a cortical portion which is often visible *in vivo* as a scale or rodlet, and is applied on the surface of a patch of material which has not yet been differentiated in living cells, but appears on fixation. It has as far as can be seen no limiting membrane, and it stains much less intensely than the cortical element. Lipoid tests show that whereas the cortical portion contains a large percentage of combined lipins there are none in the sphere substance. Examination of fixed material alone might lead one to the conclusion that this type of Golgi

body represented the remnants of a spheroidal body which had undergone partial fragmentation at the hand of the fixing agents. In fact this was the view which I took on first examining these eggs (1925). The form of the Golgi bodies as seen in the living egg however leaves no room for doubt that the rodlet is an essential part of an element which cannot be a sphere or vacuole. Nath has suggested that a sphere can under certain circumstances appear as a rodlet or crescent. It is difficult to conceive of conditions where further examination will not reveal that the true shape of such an element is spherical; and no amount of examination will reveal any further part of the crescentic rodlets of *Lumbricus* and other eggs. This statement may also be applied to the Golgi apparatus of many male germ-cells as well as to eggs. I am prepared to admit that the Golgi body may vary considerably in form from one animal to another, but the rodlet and patch type of Golgi body has been described so often in invertebrate germ-cells and also in somatic cells that I feel justified in calling in question Nath's theory of the vesicular nature of this element, the more so because of the wide divergence between his observations and my own on similar material.

Harvey (1925), Gatenby and Nath (1926), and Nath (1930) did not see the vacuome of earthworm oocytes, and Nath in particular records little success with vital dyes. Recently however Weiner (1930) and O'Brien and Gatenby (1930) have stained the vacuome with neutral red. Weiner figures the vacuome in small groups on the concave sides of the comparatively large scale-like Golgi bodies. After one to two minutes in 1/10,000 to 1/100,000 solution one or two red vacuoles appear by each Golgi body, later increasing in numbers. In a later phase of staining they wander away into the cytoplasm. On the other hand O'Brien and Gatenby, regarding the Golgi bodies as vacuoles, find the neutral red vacuoles scattered among them only after an hour's staining, and showing no positive relation to them. I have found that in well-stained oocytes, whereas occasionally a red vacuole may be seen on the concave side of a Golgi rodlet, and where two or three rodlets are lying together there may be several vacuoles among them, yet there are large

numbers of vacuoles scattered in the cytoplasm free of the Golgi bodies, and similarly many Golgi bodies free of red vacuoles. In the early phases of staining however there is a distinct relationship between the red vacuoles and the Golgi apparatus, the former appearing on the concave sides of the rodlets, and later migrating away and accumulating in little clumps in the cytoplasm. It seems highly probable that the vacuoles arise in the cell under the influence of the Golgi bodies, as a reaction of the cell to neutral red staining. Neither Weiner nor O'Brien and Gatenby could find any pre-existing counterpart of the vacuoles in unstained cells, and my own observations agree with this finding. It must therefore be concluded that they are segregation granules. The evidence goes to show that they normally arise in connexion with the Golgi apparatus just as do methylene blue granules in liver and kidney cells, the function of the apparatus being that of isolating dyes and other materials out of the cytoplasmic solution.

These observations on living cells are of interest also in connexion with the recent controversy initiated by Walker and Allen (1927) as to whether the Golgi apparatus exists in cells or is an artefact of fixation. Some support was given indirectly to their argument by the statement of Strangeways and Canti (1927) that no sign of it could be seen in the living cells of tissue cultures under dark ground observation. Against the original contention of Walker and Allen is to be set the observation—not by any means the first—of the Golgi apparatus in living unaltered cells, its form being radically changed by fixation. The further observation that these Golgi bodies which are visible by direct illumination, are not visible under dark ground illumination, except in certain circumstances, renders it improbable that this technique will reveal a Golgi apparatus that is invisible under direct lighting.

SUMMARY.

My former results (1925) have been revised, and the following are now recorded:

The mitochondria are filamentous and granular. They are present in the oogonia as a cap over one pole of the nucleus.

This cap enlarges as the oocyte grows, and finally breaks up and spreads as loose clouds over the entire cytoplasm. Under dark-ground illumination the mitochondria appear as areas showing a milky scintillation, owing to Brownian movement.

The Golgi bodies are in the form of curved rodlets tapering towards each end and having a patch of sphere material on the concave side. The rodlet only is visible in living cells. Dark-ground illumination fails to differentiate the Golgi elements from the ground cytoplasm. It is uncertain whether they are derived from one only or more than one Golgi body in the oogonium.

Droplets containing weak fat are present in all older oocytes, and in some ovaries in the younger cells, even being present in oogonia. They arise *de novo* in the cytoplasm.

The vacuome in this material is a function of the cell's reactions to neutral red, and is not present in the unstained egg. It arises in close relation to the Golgi apparatus, but in later phases of staining wanders away from it.

Nath's observations on earthworm eggs, and his theory of the vacuolar nature of the Golgi body, are discussed in detail.

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DESCRIPTION OF PLATE 11.

Fig. 1.—Nearly full-grown oocyte showing fat droplets, *f*, scattered throughout the cytoplasm, and a few Golgi bodies lying close to the nucleus at *g*. There has been a certain amount of movement of the cytoplasm during this exposure, but the perinuclear detail is not affected thereby. Note the vacuolation of the nucleolus. Zeiss 2 mm. apo., comp. oc. 7. Camera extension 8 ins.

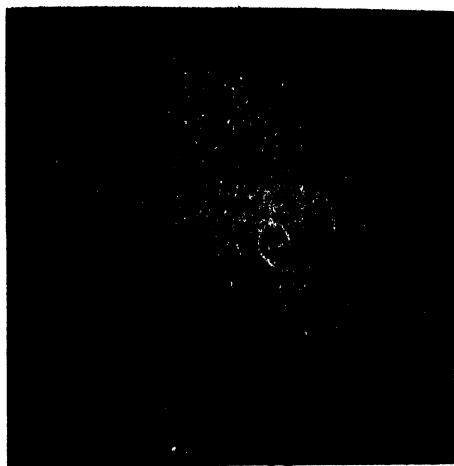
Fig. 2.—Full-grown oocyte showing scattered fat droplets, *f*, area occupied almost solely by mitochondria, *m*, and a few Golgi bodies, *g*,

lying at one side of the nucleus. Zeiss 2 mm. apo., comp. oc. 7. Camera extension 12 ins.

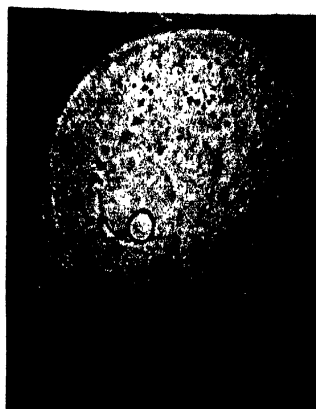
Fig. 3.—Two-thirds grown oocyte. Lettering as in previous figures. Zeiss 2 mm. apo., comp. oc. 7. Camera extension 8 ins.

Fig. 4.—Dark-ground photograph of two-thirds grown oocyte to show the highly refractive quality of the fat droplets. The general milky appearance of the cytoplasm is caused by the scattered mitochondria. Zeiss special apo. 3 mm. comp. oc. 7. Camera extension 8 ins.

These photographs are untouched, and were taken from fresh, unstained material.



f
g



f
g

3



m
g
f

2



f

4

Yolk-Formation in *Periplaneta Orientalis*.

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With Plates 12 and 13.

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I. INTRODUCTION AND PREVIOUS WORK.

It has been shown by Nath and his co-workers (18, 21, 22, and 23) that fatty yolk in certain invertebrates is formed by the deposition of free fat inside the oocyte Golgi vacuoles, and by the present writer (9) that the fatty yolk of the Tenthredinid egg is formed from the Golgi vacuoles of the oocyte and nurse-cell.

Hogben (14), for the eggs of *Periplaneta americana*, described remarkable nucleolar extrusions which migrated into the ooplasm and there gave origin to ordinary yolk. He did not investigate the part played by the Golgi elements in vitellogenesis. Consideration of the above facts led the present writer to suppose that *Periplaneta* would be a suitable form for

investigation of the phenomena associated with yolk-formation, and one which might be expected to shed light on the function of the Golgi elements. As previously pointed out by the writer (9) there is much difference of opinion at present, so that further work is necessary to establish the part played by the Golgi bodies during yolk-formation in the different groups. For although several workers ascribe a Golgi origin for the fatty yolk, others claim that the albuminous yolk is formed from these bodies.

After the present work on the yolk-formation of *Periplaneta orientalis* was commenced the writer received a reprint of Nath and Piare Mohan's (23) recent study of *Periplaneta americana*. The investigations on *Periplaneta orientalis* were continued in order (i) to compare yolk-formation in *Periplaneta orientalis* with that of the related form *Periplaneta americana*; (ii) to determine the staining reactions of the oocyte nucleolus and nucleolar extrusions; (iii) to determine by means of Feulgen's method the history of the chromatin in follicle-cell and oocyte nuclei.

Nath and Piare Mohan (op. cit.) show that the Golgi vacuoles of the oocytes of *Periplaneta americana* consist of 'an osmiophilic rim and a central osmiophobic substance'. During the growth of the egg they become distributed throughout the ooplasm and, by the deposition of fat inside them, are converted into fatty yolk-spheres. Hence the structure and behaviour of these bodies in the egg of *Periplaneta* are similar to those of the Golgi vacuoles in the other forms described by Nath and his co-workers and by the present writer. Those of *Periplaneta* appear to be slightly different, however, in that they tinge slightly with neutral red, whereas no staining was observed in those previously described by Nath and his co-workers and by the present writer. Further the Golgi vesicles (or vacuoles) of the follicular epithelial-cells of *Periplaneta* are not so intensely darkened by osmic acid as those of the oocytes, thus indicating that the former contain much smaller quantities of fat.

The albuminous yolk is formed from vacuolated nucleolar extrusions which break up into small homogeneous bodies, the latter being subsequently transformed into the yolk-spheres.

II. MATERIAL AND METHODS.

The material for this paper was obtained in November and December 1929, and in January and February 1930, from female specimens of *Periplaneta orientalis* in various stages of growth.

For an examination of the Golgi vacuoles the ovaries were dissected out in saline solution, stained in neutral red and subsequently mounted in a drop of saline or stain and examined. Ovaries were also fixed by the standard Mann-Kopsch and Kolatschev methods, while fixation for a short time in 2 per cent. osmic acid produced good results.

The studies on oocyte nucleolar extrusions and albuminous yolk-formation were carried out with ovaries dealt with by the following methods: fixed in Bouin's picro-formol and stained in iron haematoxylin; fixed in Flemming (Gatenby's modification without acetic acid) and stained in iron haematoxylin and in Auerbach's stain; fixed in corrosive acetic fixative and subsequently stained in Mann's methyl-blue eosin.

The ovaries dealt with according to Feulgen's technique were dissected out in saline solution and fixed in corrosive acetic fixative.

In all cases sections were cut 5μ in thickness.

III. OBSERVATIONS.

1. Golgi vacuoles and fatty yolk-formation.

The Golgi elements of the youngest oocytes towards the proximal end of the ovarioles were revealed by osmic methods; in Mann-Kopsch and Kolatschev preparations they appear as small dark spherical bodies situated chiefly in the vicinity of the nucleus (fig. 4, Pl. 12). With the growth of the oocyte these bodies spread through the ooplasm towards the periphery of the cell, and, at the same time, increase in size (fig. 5, Pl. 12). Later they become evenly distributed, and, in the eggs at the posterior end of the ovarioles, store up fat in their interior, increase greatly in size and become transformed into the fatty yolk-spheres (fig. 7, Pl. 12).

In ovaries fixed in 2 per cent. osmic acid the dark spheres

described above are clearly shown to consist of an osmophilic rim and a central clear substance (fig. 8, Pl. 12). In neutral red preparations clear vacuoles occupy similar positions to the dark spheres of the Mann-Kopsch and Kolatschev material, and to the dark rings shown by fixation in 2 per cent. osmic acid. Furthermore, the clear vacuoles shown in oocytes treated with neutral red are seen to develop dark rims if a few drops of osmic acid are introduced under the cover-slip and the preparation examined at intervals. Hence it is evident that these bodies are Golgi vacuoles with an osmophilic rim, similar to those previously described as occurring in the oocytes of certain other invertebrates.

The Golgi vacuoles do not stain with neutral red but remain as clear vesicles easily distinguishable from the surrounding ooplasm. At the time of their great increase in size, prior to their conversion into yolk-spheres, they are much more rapidly darkened by immersion in 2 per cent. osmic acid than are those of the earlier oocytes. This shows clearly that at a certain stage there is a great increase in the amount of fat within the vesicles, and that the fatty yolk-spheres are formed by the deposition of fat within the original Golgi vacuoles.

2. Golgi vacuoles in follicle-cells.

Golgi vacuoles were revealed in the follicle-cells of Kolatschev, neutral red and 2 per cent. osmic preparations. They are small and are situated chiefly in the vicinity of the nucleus, although many occur scattered through the ooplasm (figs. 4 and 5, Pl. 12). They do not stain with neutral red and are not rapidly darkened by immersion in 2 per cent. osmic acid.

3. Nucleolar extrusions and albuminous yolk-formation.

The nucleoli of the earliest oocytes are spherical in shape, and, as revealed by Mann's methyl-blue eosin, are faintly basophil; or consist of a central basophil portion with a slightly oxyphil margin; at this stage they may contain small vacuoles (fig. 1, Pl. 12). While the oocytes are still small and situated towards the anterior end of the ovarioles the nucleoli become irregular in

outline, are non-vacuolated and are strongly basophil in staining reaction. That the nucleoli of the older oocytes are basophil was confirmed by examination of ovaries fixed in Flemming's fixative (Gatenby's modification) and subsequently stained in Auerbach's stain. In this material the nucleoli were stained by the basic dye, but it was noted that their reaction was not so strongly basophil as in Mann's methyl-blue eosin preparations.

Soon afterwards small basophil nucleolar buds are liberated; these pass out into the nucleoplasm and towards the nuclear membrane (fig. 9, Pl. 13). Small slightly basophil bodies were observed in the ooplasm; these, although more faintly staining, closely resembled the nucleolar buds within the nucleus.

An examination of Bouin-fixed material stained in iron haematoxylin revealed the nucleolar emissions as small darkly-stained bodies situated in the nucleoplasm. Numerous small bodies, closely similar in size and staining properties, occurred scattered through the ooplasm (fig. 2, Pl. 12).

In a certain few slightly older oocytes the nucleolus appeared to be more active than in the earlier cells. In these cases the nucleolus was irregular in outline and more faintly basophil than in the other oocytes; it contained, however, several deeply basophil granules. The latter, in all probability, are buds which have not yet been liberated (fig. 10, Pl. 13).

In the older oocytes the first type of extrusion is no longer given off from the nucleolus, and, at the same time, the latter has become more spherical in outline and is vacuolated. This stage is marked by the appearance of a second type of basophil emission which has origin in the vacuoles of the nucleolus (fig. 8, Pl. 12; figs. 12 and 13, Pl. 13). These bodies pass towards the nuclear membrane and migrate to the ooplasm. They are but slightly basophil after liberation from the nucleolus and in the ooplasm become oxyphil. After extrusion they could only be distinguished with difficulty in Mann's methyl-blue eosin preparations, but in material treated with Auerbach's stain they were revealed as oxyphil bodies. It was found, however, that for the study of the nucleolar emissions ovaries stained in iron haematoxylin were the most satisfactory.

The first type of extrusion seems to disappear before the

nucleolus becomes vacuolated. This point, however, is difficult to determine with certainty, for although in many cases the ooplasm appears to become free of such bodies, in others the nucleolus develops vacuoles at an earlier stage than is usual. In a certain few young oocytes the nucleolus appeared to be breaking up to form several bodies, and, in one instance, one of the resulting masses contained small vacuoles (fig. 11, Pl. 13). It seems probable that this vacuolated portion would form the nucleolus of the later oocyte, the other parts of the original nucleolus being broken up and liberated as extrusions to the ooplasm. It would appear that the condition described above is not normal, as, in the majority of oocytes examined, the nucleoli remained whole although giving rise to numerous buds.

Several of the buds within the nucleus are vacuolated in a similar manner to the nucleolus, while many others appear to be homogenous (fig. 3, Pl. 12; fig. 13, Pl. 13). After extrusion to the ooplasm they pass towards the periphery of the oocyte, increase in size and become highly vacuolated. Later they are evenly distributed through the egg and undergo fragmentation to form small deeply-stained homogenous granules (figs. 14 and 15, Pl. 13).

The next stage is clearly demonstrated by Kolatschev material stained in acid fuchsin. The ooplasm is seen to contain a number of vesicles or globules which consist of a clear non-staining substance. These globules are apparently formed from the nucleolar extrusions which have undergone a chemical change; the presence of small dark granules within the clear substance of the former producing evidence in favour of this supposition (fig. 16, Pl. 13). The vacuoles make their appearance at the periphery of the oocytes but rapidly spread through the ooplasm. As they increase in size their staining properties change, for they now stain with acid fuchsin, and rapidly form the albuminous yolk-spheres (fig. 17, Pl. 13).

From the above account it will be seen that the first type of oocyte nucleolar extrusion disappears at an early stage. These extrusions are, in all probability, dissolved in the ooplasm, their substance, at a later stage, contributing in some way towards yolk-formation. Those of the second type appear later; they

become vacuolated and undergo fragmentation to form small dark granules which are dissolved, their substance forming clear vesicles which increase greatly in size and form the albuminous yolk-globules.

4. Feulgen's 'Nuclearreaktion'.

In material treated according to Feulgen's technique (5) chromatin was not observed in the nuclei of the oocytes, nor did the nucleolus or nucleolar extrusions contain any substance which gave the chromatin reaction. The preparations, however, did not show oogonia, consequently the present observations were confined to the growing oocytes.

The chromatin of the follicle-cells (as revealed by Feulgen's method) is in the form of granules scattered through the nuclei. Many of the granules are connected by threads which gave the chromatin reaction but were more faintly stained than the chromatin granules (fig. 6, Pl. 12).

In the follicular epithelial-cells chromatin granules were observed scattered through the nuclei (fig. 6, Pl. 12).

5. Bacteria.

The presence of bacteroid forms within the oocytes was noted. These bodies are numerous and occur at the periphery of the eggs. Similar bodies have been noted by Nath and Piare Mohan (23) in *Periplaneta americana* and more recently have been isolated and cultivated by Glaser (7). The latter worker states that the penetration of the egg by bacteria occurs after oviposition; the present writer, however, believes that in *Periplaneta orientalis* the bacteria are present in the older oocytes. However, this matter is beyond the scope of this paper.

IV. DISCUSSION.

It is evident from the above account that the behaviour of the oocyte Golgi vacuoles or vesicles of *Periplaneta orientalis* is similar to that described in the related species, *Periplaneta americana*, by Nath and Piare Mohan (23). In both species the fatty yolk-globules are formed by the deposition and accumulation of fat inside the original Golgi vesicles of the young

oocytes. Consequently, further evidence is furnished in favour of the view that the fatty yolk of certain invertebrates is formed by the transformation of the original Golgi vacuoles into yolk-spheres.

Nath (19) has demonstrated very clearly that the Golgi vacuoles in the eggs of *Culex* consist of 'an osmiophilic or argentophilic rim and an osmiophobic or argentophobic central area'. More recently the same has been shown to be true for the Golgi vacuoles observed by the writer (9) in the oocytes of certain Tenthredinids, by Nath and Piare Mohan (op. cit.) for *Periplaneta americana*, and by Nath for the earthworm, *Pheretima* (20). The present investigations show that the Golgi vacuoles of *Periplaneta orientalis* are similar in form to those of the above-mentioned types.

The work of Covell and Scott (3) on smear preparations of ventral horn and spinal ganglion cells of mice and young rabbits, stained with neutral red and subsequently treated by osmic and silver methods, is worthy of note. These workers claim to have seen the actual process of osmication and silver impregnation of granules previously stained with neutral red.

During the investigations recorded here 2 per cent. osmic acid was added to neutral red preparations of *Periplaneta* ovarioles, and subsequent examination at intervals showed that the Golgi vacuoles developed a dark rim. This demonstrated clearly that the rim of the Golgi vacuoles is osmophilic.

Gatenby (6) in a paper reviewing the work of Hirschler, Monné, Voinov, and others, shows that during the spermatogenesis of certain forms, the vacuoles become separated from the Golgi elements. Gatenby demonstrates that a similar condition exists in the male cells of *Cavia*, *Helix*, and *Abraxas*, and points out that 'the vacuole is not the Golgi apparatus, but the associate or derivative of the Golgi cortex'. He believes 'that in such examples of oogenesis as that of *Daphnia*, the Golgi element is a cortex on the vacuole'. In a recent paper on saw-fly oogenesis the present writer (9) supports Gatenby's view; furthermore, the findings of Nath and his co-workers and the present observations on the Golgi vacuoles of *Periplaneta orientalis* demonstrate clearly that during oogenesis the

Golgi cortex remains in contact with the vacuole. Hence it is evident that in the male the Golgi apparatus and vacuolar system may become separated in some way, while during oogenesis (of invertebrates at least) the Golgi cortex and vacuole remain in association.

That the Golgi bodies and vacuoles may become separated during vertebrate oogenesis is suggested by a recent note by Bhattachyra and Das (2). Small pieces of ovary from a very young pigeon, treated with neutral red, revealed the Golgi elements as spherical bodies with a clear core, and also as crescent-shaped structures. Similar bodies occupied the same position in material fixed by silver or osmic methods. The neutral red material contained 'prominent and highly refractive bodies each surrounded by two or three crescents of the nature of Golgi bodies'. These were identified as 'Golgi yolk'. Gatenby's 'vacuole' or Parat's 'vacuome' occurred as groups of vesicles, stainable with neutral red, dispersed between the Golgi elements. As the vesicles are not osmophil these workers conclude that they are 'something totally different from the Golgi bodies'.

The above account indicates that during pigeon oogenesis Golgi body and vacuole are separate as in spermatogenesis. If this be true the condition appears to be very different from that described by Hibbard for the vertebrates, *Pygosteus* (12) and *Discoglossus* (13). Until Bhattachyra and Das publish a full account of their findings no useful purpose can be served by further discussion of their claim.

The Golgi vacuoles as described by Nath and Piare Mohan for *Periplaneta americana* are slightly tinged by neutral red. In this they differ from the clear non-stainable vacuoles previously recorded by Nath (18) for the spider, *Crossopriza*, by Nath and Husian (21) for the Chilopod, *Otostigmus*, by Nath and Mehta (22) for the fire-fly, *Luciola*, by Nath (19) for *Culex* and the earthworm, *Pheretima* (20), by the present writer for Tenthredinids (9), and in the present contribution for *Periplaneta orientalis*. It seems probable, however, that this is of no great significance as the Golgi vacuoles of *Periplaneta americana* are but faintly tinged.

In a previous contribution (9) the writer drew attention to the fact that the Golgi vacuoles, which give rise to albuminous yolk in the vertebrates, *Pygosteus* (12) and *Discoglossus* (13), are stainable with neutral red until a late stage of oogenesis. Whereas those described by Nath and his co-workers and by the present writer, as giving origin to fatty yolk in certain invertebrates, do not stain but remain as clear vacuoles. Hence there is a chemical difference between the two types of Golgi vesicles even at an early stage of oogenesis. The above-mentioned facts suggest that the Golgi vacuoles in the early cells of the vertebrate ovary are similar to those described for male and other tissues, the chemical change which precedes their conversion into albuminous yolk-globules not taking place until late in the growth stage. On the other hand the Golgi vacuoles of the invertebrate egg are chemically different (as revealed by neutral red) in the earliest cells in which they have been observed.

Nath in his recent paper on *Pheretima* (where the Golgi vesicles contain fat) points out that in the amphibian, *Discoglossus*, and the invertebrate, *Carcinus* (where the Golgi elements are stated to give origin to albuminous yolk), the fatty-yolk is said to arise independently in the cytoplasm, and further that Hibbard (12 and 13) and Harvey (11) 'are dealing with "fat globules" and "fat droplets" respectively, while I am dealing with a vesicle having a definite membrane containing fat'.

The Golgi vacuoles of the follicle-cells are not so rapidly darkened in osmic acid as those of the oocytes. This agrees with Nath and Piare Mohan's findings for the Golgi vacuoles of the follicular epithelial-cells of *Periplaneta americana*. It should be noted that the cells termed 'follicular epithelium' by Nath and Piare Mohan are called follicle-cells in the present contribution.

The nucleoli of the oogonia of *Periplaneta americana*, Hogben states (14), are plasmosomes. In the oocytes, according to 'methylene blue eosin staining the plasmosome and nucleolar particles' are basophil after acid fixation, but are acidophil after fixation in Flemming (Gatenby's modification).

In *Periplaneta orientalis* all the preparations did not

reveal oogonia. In the earliest oocytes seen in Mann's methyl-blue eosin material the nucleoli are faintly basophil or consist of a basophil part surrounded by a slightly oxyphil margin; later, they become deeply basophil, as indicated by Mann's methyl-blue eosin, and by ovaries fixed in Flemming (Gatenby's modification) and subsequently stained in Auerbach's fuchsin methyl-green.

From the above account it would appear that the nucleoli of the oogonia and early oocytes of *Periplaneta orientalis* are amphiphil, or else that the nucleoli of the oogonia are oxyphil and change later as a whole from oxyphil to basophil in a manner somewhat similar to that recently described by the writer (8) for the oocyte nucleoli of a certain Tenthredinid.

In *Periplaneta orientalis* the nucleoli of many of the early oocytes were observed to contain small vacuoles; these disappear and are not connected in any way with the marked vacuolated condition observed in the older oocytes. This primary vacuolation has not been recorded for *Periplaneta americana*.

The occurrence of two types of nucleolar extrusions in *Periplaneta orientalis* agrees with Hogben's and with Nath and Piare Mohan's observations on the oocyte nucleolus of *Periplaneta americana*. It would seem, however, that in the former species the nucleolus undergoes a period of more marked activity during the liberation of the first kind of nucleolar emission. This is clearly demonstrated by the manner in which certain of the nucleoli were observed to break up into several bodies. Moreover, in one instance, one of these resulting masses was vacuolated before the cessation of the first type of extrusion. This latter condition has not been recorded for *Periplaneta americana*.

The writer's findings for the second type of nucleolar extrusion agree closely with those of Nath and Piare Mohan for *Periplaneta americana*. Many of the emissions within the nuclear membrane were observed to be vacuolated while the remainder appeared to become so on extrusion to the ooplasm. Later they break up into homogenous granules which give rise to the albuminous yolk-globules. The latter, in Kolatshev

preparations stained with acid fuchsin, at first resemble clear vesicles but rapidly undergo a change in staining properties and are stained by acid fuchsin. This last-mentioned phenomenon is not recorded by Nath and Piare Mohan for *Periplaneta americana*.

Koch (15) has shown that chromatin as revealed by Feulgen's technique, although present in the oogonia of Chilopods, is absent from the nuclei of the oocytes. He concludes that during oogenesis the chromatin undergoes a profound chemical change. In *Limnaea stagnalis* Ludford (16) finds that scarcely any chromatin is distinguishable in the oocytes. He is 'inclined to believe that the chromatin is so finely dispersed in the nucleus as to render its detection' by Feulgen's method impossible. It may be, he states, as Koch has suggested, that the chromatin of the oocytes undergoes profound chemical changes. Ludford could not detect chromatin in the oxyphil or basophil nucleoli. In the oocyte of the mouse (Ludford, 16) small granules of chromatin are present; the nucleolus, however, did not give the chromatin reaction. The present writer in a recent contribution to Tenthredinid oogenesis (10) has shown that the early oocytes of *Allantus pallipes* contain chromatin which disappears in the older cells. In the more highly developed ovarioles of *Thrinax mixta*, chromatin, although present in the nurse-cells and follicle-cells, was not detected in the oocytes. In neither species is chromatin extruded from the nuclei of the oocytes, nurse-cells, and follicle-cells. The writer pointed out that the above-mentioned observations appear to support Koch's view.

Since the findings on the chromatin of the saw-fly oocytes were sent for publication a paper on *Apanteles* by Mukerji (17) has appeared. Mukerji finds that the secondary nuclei and germ-cell determinant of the egg of *Apanteles* give a negative reaction with Feulgen's technique, and thus fail to reveal any relationship with the chromatin of the nucleus. He states that the nuclei of the early oocytes contain a few grains of chromatin, but none is present at a later stage. Before maturation the chromosomes stain purple.

The presence of chromatin granules in the young oocytes, its disappearance from the older cells, and the absence of chromatin

emissions to the ooplasm, agree with the present writer's findings for saw-flies. Mukerji states that 'it is perhaps safe to say' that the nucleolar extrusions of such forms as *Saccocirrus*, *Patella*, and *Limnaea* will be found to give a negative reaction with Feulgen's method. This is precisely what the present writer has found to be true in the case of the *Tenthredinid* nucleolar extrusions, and in the case of *Periplaneta orientalis* described in the present contribution.

In *Periplaneta orientalis* chromatin was not detected in the oocytes examined; its absence would seem to support Koch's view that during the growth of the oocytes the chromatin undergoes chemical changes. The absence of chromatin from the nucleolus and nucleolar extrusions agrees with the findings of Ludford for the mouse and *Limnaea stagnalis*, Mukerji for *Apanteles*, and the writer for *Tenthredinids*.

Faulkner (4) in a recent paper doubts the accuracy of Feulgen's method. This author states that during the early growth phases of the oocytes of the Coelenterate, *Obelia geniculata*, as revealed by observations on the living oocyte, the nucleolus elongates and fragments. 'Each fragment has been identified as a pair of homologous chromosomes indistinguishably united; later each of the bivalent elements divides in half, and the individual chromosomes are thus separated.'

Attempts to stain the nucleolar fragments by means of Feulgen's 'Nuclealreaktion' proved unsuccessful. Faulkner, however, does not consider this negative evidence of much significance as Bělár (1) says it is doubtful whether the test is specific for all nuclear phases, and Harvey (11) finds that in the oocytes of *Carcinus moenas* the chromosomes do not show the reaction after the 'bouquet' stage.

It seems that Bělár's doubt regarding the accuracy of Feulgen's technique for all nuclear phases refers to the diffuse stage, as described by Koch (15), Ludford (16), and the present writer (10). Bělár considers Feulgen's method more trustworthy than any of the former so-called chromatin reactions.

In *Carcinus* (11) the chromatin is stained up to and including the 'bouquet' stage, but after becoming diffuse it no longer gives the reaction. Clearly Harvey's observations do not support

the view that chromosomes or chromatin granules may not give a positive reaction. Furthermore, Mukerji has recently shown that the chromatin granules of the early oocytes and the chromosomes before maturation give the correct reaction. It is worthy of note that the writer has obtained a positive reaction with chromosomes in the spermatocytes of a certain *Tenthredinid*.

V. SUMMARY AND CONCLUSIONS.

1. The Golgi vacuoles and fatty yolk-formation in *Periplaneta orientalis* were studied by means of Mann-Kopsch, Kolatshev, 2 per cent. osmic acid and neutral red preparations.

2. The Golgi vacuoles of the young oocytes are situated in the vicinity of the nucleus; later they pass to the periphery of the cell. In the older oocytes, towards the posterior end of the ovarioles, they become evenly distributed in the ooplasm, store up fat, increase greatly in size, and give rise to the fatty yolk-spheres. In the older oocytes they darken much more rapidly in 2 per cent. osmic acid.

3. In neutral red preparations clear non-stained vacuoles are seen to occupy similar positions to those of the dark bodies of the osmic preparations; on introducing a few drops of 2 per cent. osmic acid under the cover slip the vacuoles develop an osmophilic rim. These Golgi vacuoles are not stained by neutral red.

4. In 2 per cent. osmic acid preparations the Golgi vacuoles are seen to consist of an osmophilic rim and a central clear substance.

5. The Golgi vacuoles of the follicle-cells are similar to those of the egg, except that they do not increase greatly in size and are not so rapidly darkened in 2 per cent. osmic acid.

6. The nucleoli of the early oocytes are spherical in shape and are amphiphil or slightly basophil in staining reaction; they may contain small vacuoles. In slightly older oocytes the nucleoli are non-vacuolated; they become strongly basophil, irregular in outline, and, at the same time, give rise to emissions which pass through the nuclear membrane to the ooplasm, where they ultimately disappear. In a certain few oocytes the nucleolus

was seen to have broken up into several masses, some of the latter, in all probability, fragmenting to form nucleolar extrusions. In a certain oocyte one of the masses was observed to be vacuolated before the first type of extrusion had ceased.

7. In the more highly developed oocytes the first type of nucleolar emission ceases, and the nucleolus becomes more spherical in outline. Numerous vacuoles appear which give origin to nucleolar extrusions. The latter become vacuolated, either before extrusion through the nuclear membrane, or later in the ooplasm.

8. The second type of nucleolar extrusions pass to the periphery of the egg. Later they become evenly distributed in the ooplasm, where they fragment to form homogeneous granules. The latter form clear spheres (Kolatschev material) which rapidly increase in size to form the albuminous yolk-globules.

9. Chromatin was not observed in the oocyte nuclei, nucleoli, or nucleolar extrusions (Feulgen's technique). The chromatin of the follicle-cells is in the form of granules connected by threads (which give the chromatin reaction). The chromatin of the follicular epithelial-cells was observed as granules scattered through the nuclei.

10. Bacteroid forms were observed in the ooplasm at the periphery of the older oocytes.

11. The method of yolk-formation is similar to that of *Periplaneta americana* as described by Nath and Piare Mohan.

12. The writer's conclusions regarding the shape and character of the Golgi vacuoles agree with the findings of Nath and his co-workers and with the former conclusions of the present writer for oocyte Golgi vacuoles.

VI. ACKNOWLEDGEMENTS.

The work recorded in this paper and in a series on the Tenthredinidae (Gresson, 8, 9, and 10) has been prosecuted under Professor A. D. Peacock in the Zoology Laboratory of University College, Dundee, and has been assisted by a grant from the Royal Society of London. I have pleasure, therefore, in giving acknowledgements and thanks for these facilities.

ADDENDUM.

Since this paper was completed Miss M. 'O'Brien and Professor J. Brontë Gatenby have published a note on the oögenesis of *Lumbricus* (*Nature*, vol. 125, p. 891, 1930). These workers show that in the oocytes of *Lumbricus*, globules, which stain with neutral red, are present; these globules, however, are not related to the Golgi elements. They state that 'it does not seem possible entirely to dismiss the idea that these globules might be segregation vacuoles and not pre-existing structures'. They succeeded in staining a 'vacuome' in coelomic epithelial cells and in nerve-cells.

The present writer has recently carried out further examinations of *Periplaneta* ovarioles stained in neutral red. In no case were vacuoles or granules stainable by neutral red detected, although the same sample of neutral red stained the vacuolar system of male germ-cells and oocytes of *Helix*.

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EXPLANATION OF PLATES 12 AND 13.

LETTERING.

Ay, albuminous yolk; *Ay*¹, clear spheres formed from nucleolar extrusions; *cgr*, chromatin granules; *Fc*, follicle-cell; *Fe*, follicular epithelium; *Fy*, fatty yolk; *G V*, Golgi vacuole; *Nu*, nucleolus; *Nue*, nucleolar extrusion; *Nuf*, nucleolar extrusion undergoing fragmentation; *oo*, ooplasm.

PLATE 12.

Fig. 1.—Early oocyte nucleus at anterior end of ovariole; nucleolus contains a few small vacuoles. Mann's methyl-blue eosin.

Fig. 2.—Young oocyte. Nucleolar emissions in nucleus and in the ooplasm. Iron haematoxylin.

Fig. 3.—Nucleus of older oocyte. Nucleolus vacuolated; numerous nucleolar emissions in nucleus and in ooplasm. Iron haematoxylin.

Fig. 4.—Young oocyte. Golgi vacuoles in oocyte and follicle-cells. Kolatschev.

Fig. 5.—Older oocyte. Golgi vacuoles have increased in size and are situated towards the periphery of the egg. Kolatschev.

Fig. 6.—Follicle-cells and follicular epithelium. Chromatin granules in follicle-cell nuclei; many of the granules are connected by threads. Chromatin granules in follicular epithelial cell nuclei. Feulgen's technique.

Fig. 7.—Golgi vacuoles, fatty and albuminous yolk-spheres. Mann-Kopsch.

Fig. 8.—Golgi vacuoles showing osmophilic rim. 2 per cent. osmic acid.

PLATE 13.

Fig. 9.—Showing nucleolus and first type of nucleolar extrusion. Mann's methyl-blue eosin.

Fig. 10.—Nucleolus appears to be breaking up into numerous buds. Mann's methyl-blue eosin.

Fig. 11.—Nucleolus breaking up; one of the resulting masses contains small vacuoles. Mann's methyl-blue eosin.

Fig. 12.—Older oocyte showing vacuolated nucleolus and second type of nucleolar extrusion. Mann's methyl-blue eosin.

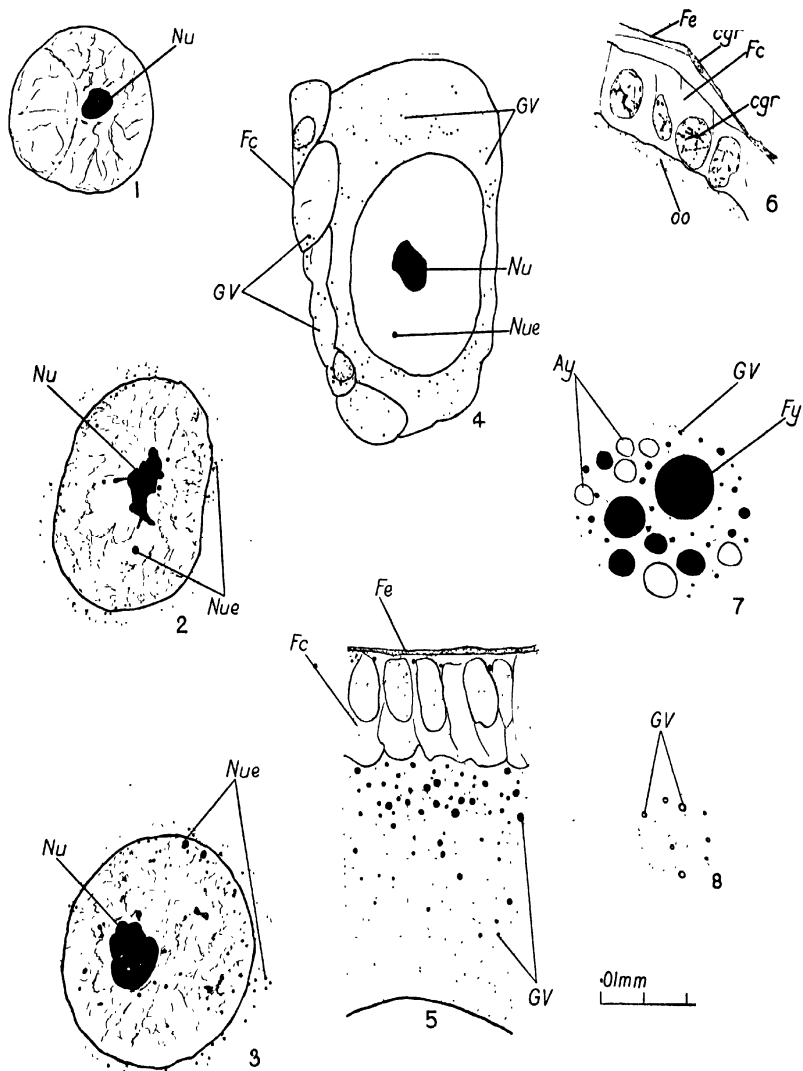
Fig. 13.—Showing vacuolated nucleolus and vacuolated nucleolar extrusions in the nucleoplasm. Mann's methyl-blue eosin.

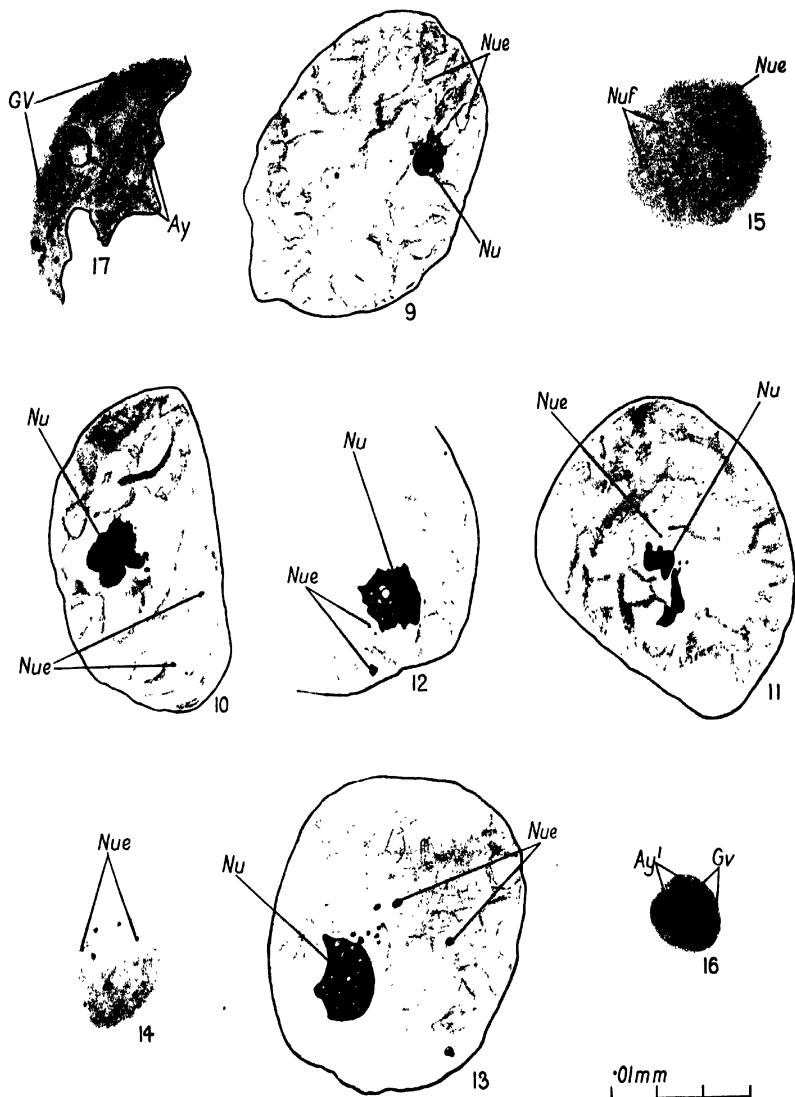
Fig. 14.—Showing vacuolated nucleolar extrusions in the ooplasm. Iron haematoxylin.

Fig. 15.—Nucleolar extrusions undergoing fragmentation in the ooplasm. Iron haematoxylin.

Fig. 16.—Clear spheres formed from nucleolar extrusions. Kolatschev.

Fig. 17.—Showing Golgi vacuoles and albuminous yolk-globules. Kolatschev.





The Cranial Characters of the Brevicipitid Genus *Cacosternum* (Boulenger).

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With 12 Text-figures.

THE present paper is a continuation of my work on the cranial characters of the two South African Brevicipitid genera lacking the claviculo-procoracoidal arch in the pectoral girdle. The genus *Phrynomerus* (Noble) was discussed in a previous paper in this Journal (vol. 73), to which the reader is referred for details of technique, which was, however, slightly varied for purposes of the present research, inasmuch as double bulk-staining was resorted to. The nuclear stain, haemalum, was applied in the usual way, and after all traces of alum used for differentiation were washed out, the object was bulk-stained for twenty-four hours in a strong aqueous solution of Bismarck brown and differentiated in ordinary tap-water for forty-eight hours. I have not yet experimented with bulk-staining for connective tissue and muscle; stains recommended for this purpose are fuchsin and light green or eosin respectively. The use of van Gieson in conjunction with Bismarck brown is considered by many workers to lead to confusing results, so that pure fuchsin is recommended for connective tissue and for decalcified bone. All material was decalcified with Ebner's solution in preference to nitric acid, which is, however, used with great success for the same purpose by Professor Stadtmüller of Göttingen, according to a personal communication from him. The material of *Cacosternum böttgeri* was kindly supplied by the Director of the Transvaal Museum; *Cacosternum capense* was collected by the author on the Stellenbosch Flats after the first rains in May 1928. The eighteen specimens collected are the only ones known besides the type

specimens collected by Mr. Rose of Cape Town. Three specimens of *Cacosternum namaquense* were collected by Dr. Herre of Stellenbosch University during the phenomenal Namaqualand rains of 1929; but owing to the rarity of the material, this species was not microtomed. Mr. Hewitt has recently upheld the species and recorded the existence of two specimens referable to it in the collection of the South African Museum.

REVIEW OF THE EXISTENT LITERATURE ON THE GENUS.

The genus *Cacosternum* was first described by Boulenger (1887, page 51). The new genus was referred to the Engyostomatidae and the following osteological details were enumerated as being characteristic of it: palate toothless, without dermal ridges; tympanum hidden; no procoracoids; coracoids slender; sternum extremely small, cartilaginous; diapophyses of sacral vertebrae strongly dilated. The presence of strong subarticular tubercles of the fingers and toes, also present in *Phrynomerus*, was mentioned. The first South African species to be described was *C. böttgeri* in Boulenger's catalogue of 1882, where it is referred to as *Arthroleptis böttgeri* (p. 118). Werner (1910) described a new species of *Cacosternum*, *C. namaquense*. Boulenger (1906-9) first called *C. böttgeri* by the name now generally used. No cranial characters are mentioned in this work. Valuable osteological data are supplied by Hewitt (1911) from which I select the following: (p. 215) 'maxillary and premaxillary teeth present, no vomerine teeth, palate without dermal ridges, tympanum hidden. In the skull a fronto-parietal foramen is present and the fronto-parietals are but feebly ossified'. Hewitt then regarded *Cacosternum* as allied to the *Dyscophids*, on account of the presence of teeth. Andersson (1911) merely notes the occurrence of *Phrynomerus* and *Cacosternum* in British East Africa, where the Swedish Zoological Expedition made a collecting tour in 1911. Methuen (1913, p. 123) classifies *C. böttgeri* under the sub-family *Dyscophinae* of the Engyostomatidae, probably on account of the presence of teeth on the maxilla. No cranial characters are mentioned. Hewitt (1919) discusses

in detail the affinities of the *Cacosternum*-*Anhydrophryne* group (pp. 184-7) and concludes that the absence of procoracoids is of greater importance than the presence of maxillary teeth and stresses the affinities with the Malagasy *Dyscophids*. The presence of a fronto-parietal fontanelle in *Breviceps* and *Cacosternum* is not considered by the author as of great systematic importance. That part of Noble's work (1922) dealing with *Cacosternum* and its allies is of extreme importance, as it discards the toothed '*Dyscophidae*' as an independent family and classes them with allied toothless forms under the new family *Brevicipitidae*. Noble's paper of 1924 contains interesting speculations on the origin of the Ethiopian *Brevicipitidae*, which are referred to the older African fauna which flourished when the mainland was still connected with Madagascar (pp. 277 and 278), and suggests possible affinities between the *Cacosternum*-*Anhydrophryne* group and the Malagasy *Anodonthyla*, the affinities of which genus are further discussed by Noble (1926, pp. 2-4), the conclusion arrived at being that *Anodonthyla* is derived from a form with divided, dentigerous vomer. In 1926 Hewitt contributed valuable information regarding the morphology and systematics of the genus *Cacosternum*: *C. capense*, the new Cape species described by the same author in 1925, was shown to possess a divided coracoid, which may be of great value in a comparison of *Cacosternum* and *Phrynomerus*. Werner's species (1910) of *C. namaquense* was upheld and found to be allied to *C. capense*, from which it differs mainly in being toothless. A new sub-species of *C. böttgeri*, *C. böttgeri albiventer* was described. *C. namaquense*, of which Werner's specimens were supposed to be the only ones extant, was rediscovered by Dr. Herre at !Koeboes, Richtersfeld, Namaqualand. The three specimens collected in October 1929 agree in every detail with those described by Werner, who collected them at Steinkopf. The new specimens are in the Department of Zoology of the University of Stellenbosch. Noble (1926 a, p. 4) again refers to the possible relationship of *Cacosternum* and *Anhydrophryne*, and of the latter genus with the genera *Arthroleptis* and *Arthroleptella*, without,

in the opinion of the author of this paper, making good his point. Nieden (1926, p. 11) groups *Cacosternum* under the Engystomatidae and is therefore not aware of the discovery of maxillary teeth in the genus by Hewitt (1911). Power published a paper dealing with *Cacosternum* in 1927. On p. 250 he comes to the remarkable conclusion that '*Cacosternum* has bufonid affinities', but bases his argument solely on the pattern of the chitinous tooth-rows and the position of the anus. The present author published a paper (1929 *a*) on the macroscopic development of *C. capense*, whose spawn and larvae are extremely common round Stellenbosch, and is of opinion that the larvae are in appearance and in habit as unlike *Bufo* larvae as they possibly can be, and resemble much more closely those of the genus *Pyxicephalus*. Mr. Rose, the discoverer of *C. capense*, gave a preliminary account of its habits and life-history in 1926 (p. 437). The latest paper up to date, referring to *Cacosternum*, is that of Noble (1927, pp. 116 and 117), in which the following conclusions are reached: (1) The pectoral girdles of *Cacosternum* and *Phrynomerus* agree closely; (2) the larvae of the former genus are ranid and unlike those of *Phrynomerus*; (3) *Anhydrophryne* was evolved from *Arthroleptella*, from which it differs by the absence of a clavicle; (4) the close relationship of *Cacosternum* and *Anhydrophryne* is stressed; (5) *Cacosternum* is possibly also derived from *Arthroleptella* but is in any case 'closely related to ranids and not to other Brevicipitids'. In a paper read before the British Association at the Cape Town Session, the author of this paper has fully described the non-aquatic life-history of *Arthroleptella* and has proved that the genus is perfectly ranid. Contrary to what Noble presupposes, it has no clavicle, but a procoracoid only. It was suggested that *Arthroleptella*, *Cacosternum*, and *Anhydrophryne* might have to be removed to the Ranidae.

RESULTS OF OWN INVESTIGATIONS.

It is proposed in the following account to discuss chiefly those features of the cranial characters of the genus *Cacosternum*

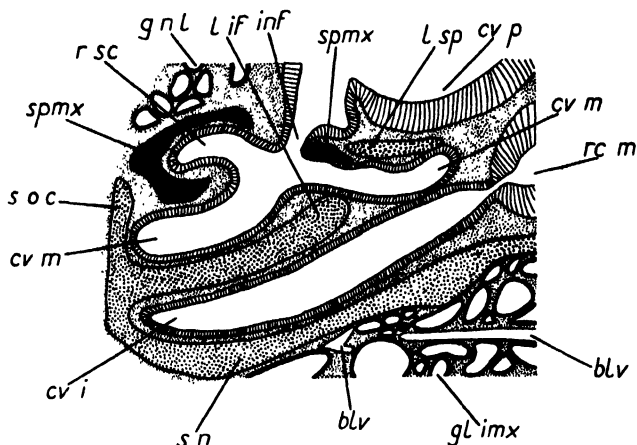
in which it differs from *Phrynomerus*, the other South African genus lacking a procoracoid. The following description applies in the main to *Cacosternum capense*: the difference between it and the smaller species, *C. böttgeri*, will duly be noted. *C. namaquense* was not sectioned as the material is too rare.

THE OLFATORY CAPSULE.

Both cartilagines praenasales are present as in *Phrynomerus*; the superior is short, the inferior long and flexed beneath the solum nasi, and forms the main support for the premaxilla. It is imbedded in the tubules of the large glandula intermaxillaris. The same conditions prevail in *C. böttgeri*. The two vestibular 'Wülste' are both present and the plica obliqua is blunt and short and suspended from the cartilago obliqua as in *Phrynomerus*, not from the tectum nasi as in *Rana*; the same applies to *C. böttgeri*. The recessus sacciformis, described by Gaupp for *Rana*, was found to be absent in *Phrynomerus*. In *Cacosternum* it is, however, present, but its anatomical relations are not the same as in *Rana*. Gaupp described the organ on pages 625 and 633 of the third volume of the 'Anatomie des Frosches' (1904). On page 625 we read: 'Der Wulst [i.e. the one taking the place of the cartilago alaris] ist von unten und hinten her gewissermassen unterminiert durch den Recessus sacciformis, der hinten in die Vestibularnische übergeht, medial- und ventralwärts mit dem Infundibulum und dem Cavum medium zusammenhängt.' Additional details are furnished on p. 633: 'Lateral von diesem Wandwulst stülpt sich ein schlaffwändiger Schleimhautblindsack, der Recessus sacciformis, nach vorn und oben hin vor, der seinen Ausgang von der erwähnten Kommunikationsspalte und im Anschluss daran (nach vorn hin) auch noch von der lateralen Kante des Cavum medium nimmt.' In *Phrynomerus* an evagination of the infundibulum and cavum medium is absent, but a sac-like recess is present in *Cacosternum*, although it does not undermine the large 'Wulst', is short and wide instead of high and narrow as in *Rana* (see Gaupp, loc. cit., fig. 140, p. 627), and does not communicate with the vestibule. The

recessus in *C. capense* is figured in Text-fig. 1, from which it will be seen that it originates from both infundibulum and cavum medium; it does *not* represent the point of communication of the ductus nasolacrimalis with the cavum medium, as the

TEXT-FIG. 1.



Transverse section through the three narial cavities of the left side in *Cacosternum capense*. *bl.v.*, blood-vessel; *cv.i.*, cavum inferius; *cv.m.*, cavum medium; *cv.p.*, cavum principale; *gl.imx.*, glandula nasalis intermaxillaris; *g.n.l.*, glandula nasalis lateralis; *inf.*, infundibulum; *l.if.*, lamina inferior cristae intermediae; *l.sp.*, lamina superior cristae intermediae; *rc.m.*, recessus medialis; *r.sc.*, recessus sacciformis; *s.n.*, solum nasi; *s.o.c.*, side of olfactory capsule; *spx.*, septomaxillary.

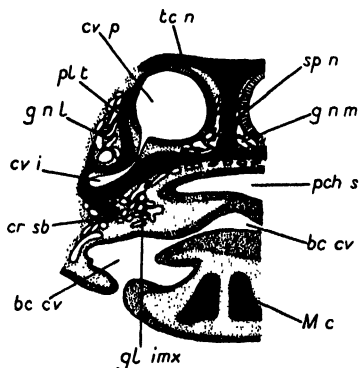
latter receives the duct at its blind, lateral division, after the recessus sacciformis has disappeared from sections. It will be seen from the figure that the recess is surrounded by the septomaxillary bone, which forms a capsule for it. In *C. böttgeri* the recess has exactly the same disposition as in *C. capense*.

In *Phrynomerus* two remarkable prechoanal sacs were described (loc. cit.); they were shown to be derived from an unpaired prechoanal sac in the young form. The apparatus is easily derived from a fusion of the two 'Gaumenleisten' (Gaupp) or palatal ridges. In *Cacosternum*, Text-fig. 2, there is an

unpaired prechoanal sac as in the young *Phrynomerus*, but the choanae no longer open into, but just beyond it. In *C. böttgeri* the sac was filled with dense mucous material.

The premaxilla and maxilla have the usual relations with neighbouring structures, but are not separated palatally by the ventrally flexed crista subnasalis as in *Phrynomerus*. They are moreover toothed: the teeth consist of a functional

TEXT-FIG. 2.

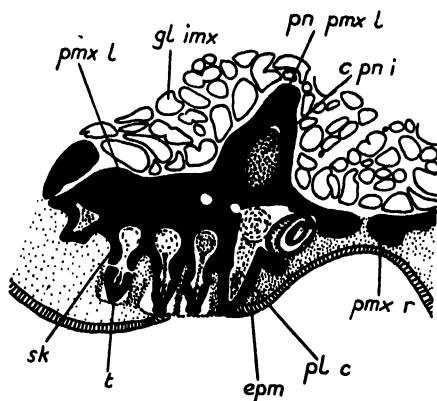


Transverse section through the head of *Cacosternum capense* in the anterior region of the olfactory capsule. *bc.cv.*, buccal cavity; *cr.sb.*, crista subnasalis; *gn.m.*, glandula nasalis medialis; *M.c.*, Meckel's cartilage; *pch.s.*, prechoanal sac; *pl.t.*, planum terminale; *sp.n.*, septum nasi; *tc.n.*, tectum nasi. Other abbreviations as for Text-fig. 1.

row (acrodont), and a few additional rows waiting to take the place of the former. The premaxillary teeth of *C. böttgeri* are sketched in Text-fig. 3; they have the normal histological structure of anuran teeth as described for *Rana* by Krause (1923), and were discovered in the genus by Hewitt (1911). *C. namaquense* differs from the other species in being toothless. The vomer in *C. capense* is a very small bone investing the edge of the solum nasi region of the choana. There is no considerable prolongation of the bone into the narial cavity, as *Cacosternum* lacks the enormous cartilaginous axis of the eminentia olfactoria, which the vomer invests in

Phrynomerus. *C. böttgeri* has a comparatively large vomer, its size being due to the extent of the squame investing the ventral surface of the solum nasi. The bone encapsules a few tubules of the glandula intermaxillaris. In both species the vomer is entirely edentulous, and separated from its fellow on the other side by fibrous connective tissue. In *C. capense* the two bones approach each other most closely. The palatine is widely separated from the vomer, so that no vomero-

TEXT-FIG. 3.



Section through the left premaxilla of *Cacosternum böttgeri*. *c.pn.i.*, cartilago praenasalis inferior; *epm.*, epithelium of buccal cavity; *pl.c.*, pulp cavity of tooth; *pmx.l.*, left premaxilla; *pmx.r.*, right premaxilla; *pn.pmx.l.*, prenasal portion of left premaxilla; *sk.*, socket of tooth; *t.*, tooth. Other abbreviations as for previous figures.

palatine is formed as in *Phrynomerus*. The bone invests the ventral surface of the processus antorbitalis as in *Rana* and is of course quite edentulous. The septomaxillary is topographically confined to the lamina superior cristae intermediae and, as stated above, encapsules the recessus sacci-formis. As in *Phrynomerus*, the bone terminates in front of the planum terminale of the cartilago obliqua. The relation of nasals and os en ceinture in *Cacosternum* was first remarked upon by Noble (1926), who pointed out that the

nasals are very small bones appearing on the sides of the large os en ceinture, which latter is prolonged into the tectum nasi. At their maximal width a nasal in *Cacosternum* covers about one-sixth of the tectum nasi, so that two-thirds of the latter are exposed; laterally the nasal does not articulate with the maxilla as in *Rana*. This peculiarity is also met with in *Phrynomerus*, but the posterior bay in the nasal is absent.

For the rest, the region of the olfactory capsule and associated structures differs very little from the well-known type met with in the frog. The absence of an enlarged eminentia olfactoria and associated cartilaginous axis encountered in *Phrynomerus* is probably to be interpreted as lack of specialization in this direction.

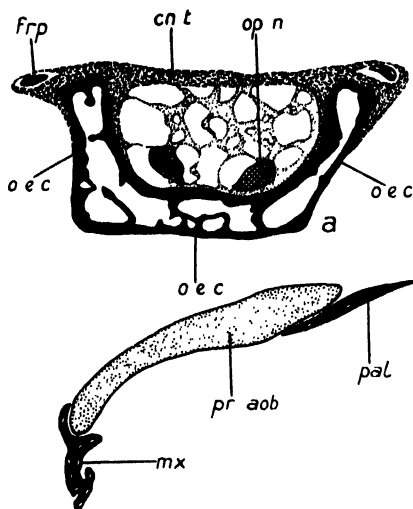
OSSIFICATIONS IN THE SPHENETHMOID REGION.

In neither of the two species sectioned is the os en ceinture ossified so far forwards as one might expect. The first traces of the bone are met with in the nasal septum at the level of the choana and the posterior limits of the vomers. This is exactly the state of affairs in *Rana* as well. The ossification spreads to the tectum nasi at the region of the anterior limits of the processus antorbitalis, but the ventral portion of the narial skeleton only begins to show signs of ossification towards the posterior region of the processus antorbitalis, which is not partially incorporated into the os en ceinture as in *Rana*. These features are drawn in Text-fig. 4, *a*. In the region of the roots of the olfactory nerves (Text-fig. 4, *b*) the os en ceinture appears upon transverse section as a densely ossified trough with no traces of cartilage in its walls. In *Phrynomerus* the free dorsal ends of the trough persist as cartilage, which also constitutes the central portion of its bottom. More posteriorly these three tracts of cartilage also appear in *Cacosternum*; it is interesting to note that the appearance of the mid-ventral tract indicates the presence of a posterior ventral notch in the os en ceinture. This is not represented in *Rana*. The anterior division of the os en ceinture in *C. böttgeri* is similar in appearance to that of *C. capense*, but the posterior portion is like that of *Phrynomerus*, inasmuch as no complete

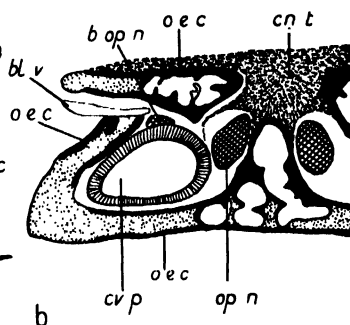
ossification occurs, the three tracts of cartilage persisting as in *Phrynomerus*.

It will be convenient to leave the discussion of the other cartilage bones to a later stage and to consider forthwith the fronto-parietal bones and the so-called fronto-parietal

TEXT-FIG. 4 a.



TEXT-FIG. 4 b.



4 a: Transverse section of the skull of *C. capense* cut posterior to the antorbital process.

4 b: Transverse section through the region of the antorbital process of *C. capense*.

b.op.n., branch of the optic nerve; *cn.t.*, connective tissue; *frp.*, fronto-parietal; *mx.*, maxilla; *o.e.c.*, os en ceinture; *op.n.*, optic nerve; *pal.*, palatine; *pr.aob.*, processus antorbitalis. Other abbreviations as for previous figures.

fontanelle. Hewitt (1911) remarked upon the weak ossification of the fronto-parietals and called attention to the presence of the fontanelle in *C. böttgeri*. These two features are also met with in *Cacosternum capense*. The tips of the fronto-parietals are figured in Text-fig. 4, a; they are imbedded in tough connective tissue which forms the roof of the brain case. The bones do not increase greatly in size in the interorbital region but are always joined by a wide expanse of connective

tissue as in *Phrynomerus*; they attain to their maximal size in the region of the anterior limits of the otic capsule, but even so they do not touch in the middle line, although they are considerably more strongly developed than in *Phrynomerus*. Conditions in *C. böttgeri* correspond very closely to those in *C. capense*, but the fronto-parietals are even smaller and hardly project from the otic capsule into the connective-tissue cranial roof. This is almost exactly similar to the *Phrynomerus* pattern.

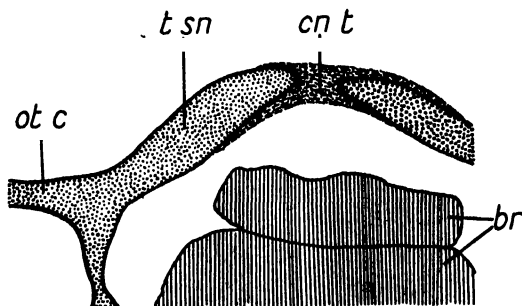
The pro-otic:—The optic foramen is bounded anteriorly by cartilage, but its postero-dorsal margin is formed by the pro-otic bone. Exactly the same conditions prevail in *C. böttgeri*. All ossification of the septum dividing the cavity of the otic capsule from the brain-cavity disappears before the foramen acusticum and the foramen endolymphaticum are sectioned, whereas in *Rana* and *Phrynomerus* the anterior boundary of the former foramen is part of the pro-otic. Posterior to the two foramina mentioned, the otic capsule is almost entirely cartilaginous. Weak ossification of the capsule was also a feature of *Phrynomerus*, in which the supra- and infra-cristal ossifications were likewise absent. *C. böttgeri* has the same type of otic capsule as *C. capense*, except that in the latter species the posterior division contains much more persistent cartilage than in the former.

The cranial roof consists of fibrous connective tissue in the orbital region; towards the anterior boundary of the otic capsule the tip of the taenia tecti medialis is sectioned; when this spreads laterally and passes into what should represent the taenia tecti transversalis, the cartilaginous cranial roof shows no more foramina, so that as in *Phrynomerus* and *Arthroleptella* (de Villiers, 1929) the parietal foramen is absent and the taenia tecti transversalis is confluent with the tectum synoticum. It is of course also possible to consider this state of affairs to be due to the absence of the transverse taenia; but comparative data are not available; Gaupp's original description referred to the European *Rana fusca*. A remarkable feature of the tectum synoticum of *Cacosternum* is its weak development towards the foramen magnum, where it possesses a deep

notch filled with connective tissue (Text-fig. 5). The exoccipitals are comparatively small; ventrally between them a portion of the planum basale persists as cartilage, which, however, shows no traces of a notochord.

The parasphenoid stretches from the mid-ventral division of the os en ceinture to the ventral occipital region, is a dagger-shaped bone as in *Rana* and, as in that genus, possesses two posterior notches separating the longitudinal portion from the

TEXT-FIG. 5.



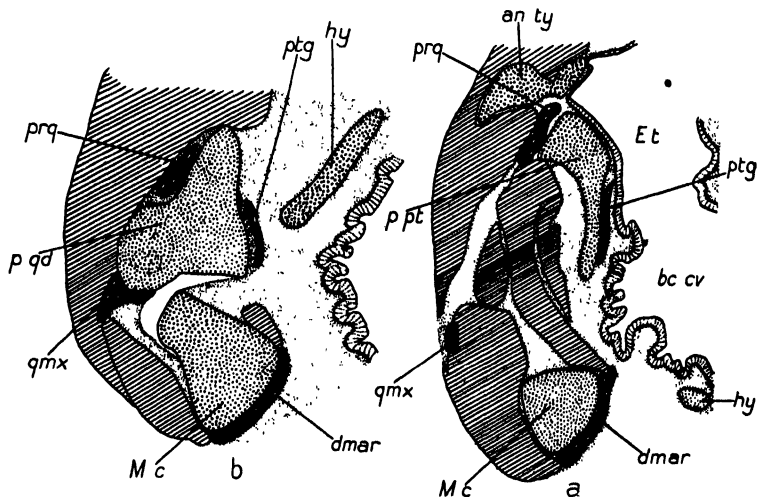
Transverse section through the posterior limits of the tectum synoticum of *C. capense*. *br.*, brain; *ot.c.*, otic capsule; *t.sn.*, tectum synoticum. Other abbreviations as for previous figures.

lateral ones, which attain their greatest size at the level of the posterior region of the otic capsule. The cartilaginous cranial roof lacks a taenia transversalis in *C. böttgeri* also. The exoccipitals are comparatively large and the tectum synoticum has no bay dorsal to the foramen magnum. The parasphenoid is dagger-shaped, but posteriorly the longitudinal portion is not marked off from the lateral ones by deep notches.

The morphology of the quadratimaxillary and paraquadrates was reinvestigated by the author in *Arthroleptella* (1929) and *Phrynomerus* (Quart. Journ. Micr. Sci., vol. 73). *Cacosternum* is most remarkable in possessing a totally unossified quadrate cartilage, whereas in other Anuran genera, including *Rana*, as described by Gaupp, the quadrate ossifies perichondrally and enchondrally and fuses with the

quadratomaxillary. The relations of the above-mentioned bones in *Cacosternum* are sketched in Text-fig. 6, *a* and *b*; *a* represents a transverse section of the suspensorial region of the head and passes through the Eustachian tube. The quadratomaxillary occupies a position external to the chewing muscles, but

TEXT-FIG. 6 *a* AND *b*.



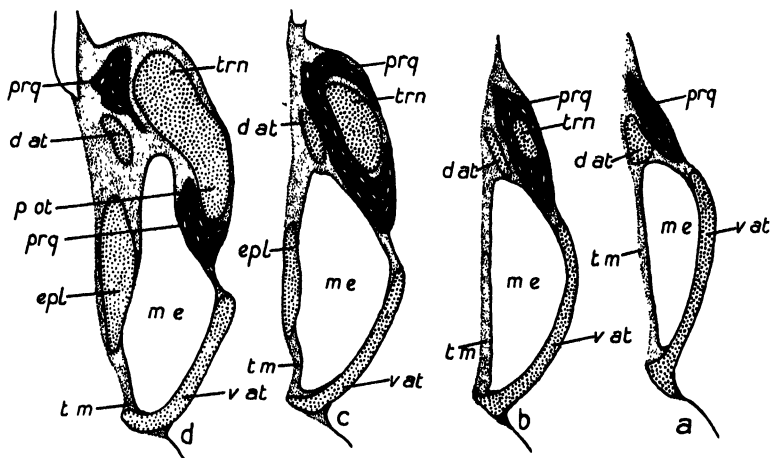
Transverse sections through the articular region of the skull of *C. capense* to show the relations of the quadratomaxillary to the rest of the skeleton (muscles are ruled). *an.ty.*, annulus tympanicus; *dmar.*, dermarterial; *Et.*, Eustachian tube; *hy.*, hyale; *p.pt.*, processus pterygoideus; *p.qd.*, pars quadrata processus pterygoidei; *prq.*, paraquadrata; *ptg.*, pterygoid; *qmx.*, quadratomaxillary. Other abbreviations as for previous figures.

more posteriorly it gradually shifts in between these latter, and appears as an investing bone of the quadrate cartilage. It is, however, easily distinguishable from dermal bones by the fact that it is not separated from the quadrate cartilage by a connective-tissue lamella (Text-fig. 6, *b*). The bone shoots a few diminutive rootlets into the invested cartilage, but the characteristic cartilaginous structure of the latter is in no way affected. Exactly the same conditions prevail in *C. böttgeri*.

The paraquadrata is a comparatively small bone in

Cacosternum and is first encountered in sections at the level of the closure of the optic foramen; it then lies external to the temporal muscle and does not articulate anteriorly with any bone as it does in *Pyxicephalus adspersus*. Upon reaching the transitional region between the processus oticus and the crista parotica, the paraquadrates form a bony sheath

TEXT-FIG. 7 *a, b, c, d.*



Consecutive transverse sections through the tympanic region of *C. capense*. (It is impossible to locate definitely the common boundary of the crista parotica and the processus oticus. This region is therefore labelled *trn.*, transitional cartilage.) *dat.*, dorsal portion of annulus; *epl.*, extrapleural enlargement of the pars externa plectri; *me.*, middle ear; *p.ot.*, processus oticus; *tm.*, tympanic membrane; *v.at.*, ventral portion of annulus. Other abbreviations as for previous figures.

for the latter. For a considerable stretch no connective tissue is present to separate the bone from the cartilage, which therefore simulates perichondral cristal ossification. Enchondral ossification of the cartilage is absent. The paraquadrates are typically triradiate as in all *Anura* known to me; the two transverse posterior rays are normal investing bones of the quadrates cartilage and crista parotica. The relations of the paraquadrates, crista parotica, processus oticus and annulus tympanicus are sketched in Text-fig. 7, *a, b, c, d.* *C. böttgeri*

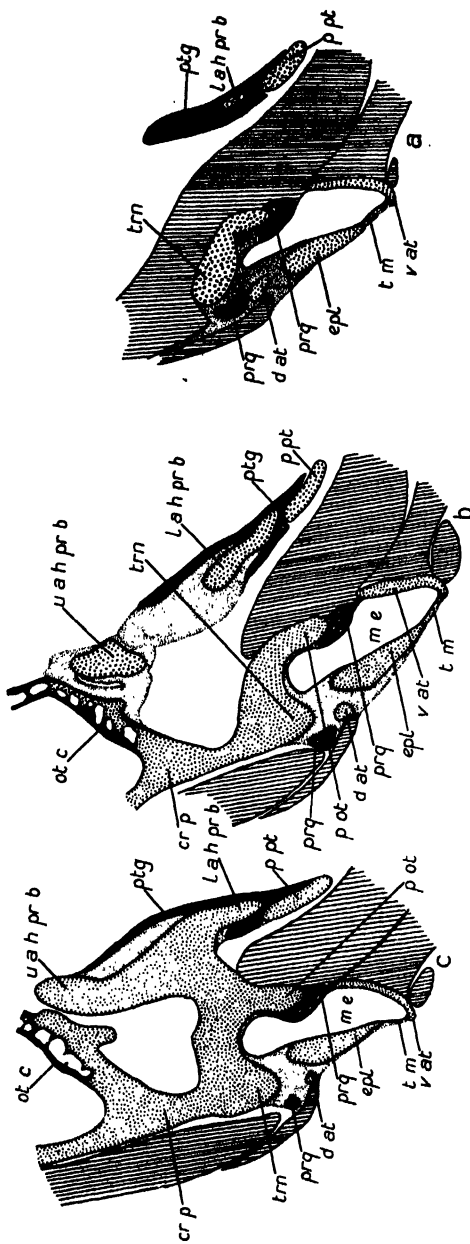
does not differ very much from *C. capense*, but a stage corresponding to Text-fig. 7, *b*, is missed out, so that the paraquadrates form a groove, and not a sheath for the transitional cartilage.

The pterygoid invests the dorsal and inner surfaces of the processus pterygoideus and possesses in its dorsal portion a well-developed marrow cavity. On the whole it may justly be maintained that the pterygoid is much more strongly developed dorsal to the processus pterygoideus than in *Rana*. The anterior portion of the processus basalis is entirely ensheathed by the pterygoid, which is not in this region separated from the cartilage by connective tissue (Text-fig. 8, *a*). The two anteriorly directed horns of the processus basalis are sectioned in Text-fig. 8, *b*; the tip of the lower touches the pterygoid bone, but its upper division and the dorsal anterior horn of the processus basalis are again separated from the investing pterygoid by connective tissue. Text-fig. 8, *b*, shows the junction of the processus oticus and crista parotica, and Text-fig. 8, *c*, marks the fusion of this cartilage mass with the processus basalis. Posteriorly the pterygoid persists as a diminished bone on the inner surface of the pars quadrata. As far as comparative data are available, these conditions agree topographically quite well with those in *Rana*. *C. böttgeri* agrees with *C. capense* even in the minutest details, the only point of difference being the circumstance that the lower anterior horn of the processus basalis is not entirely ensheathed by the pterygoid.

THE AUDITORY APPARATUS

Typical transverse sections through the anterior region of the annulus tympanicus and middle ear are shown in Text-fig. 7, *a* and *b*; since the annulus has the form of a disk with a central perforation, it will appear in transverse section as two cartilages, the ventral of which is the larger on account of the eccentricity of the perforation referred to above. The pars externa plectri begins to appear in Text-fig. 7, *c* as a longish cartilage imbedded in the mesodermal portion of the tympanic membrane. Such an extrapleural enlargement of the pars externa was also encountered in *Phrynomerus*. Text-fig. 8, *a*, *b*, and *c*,

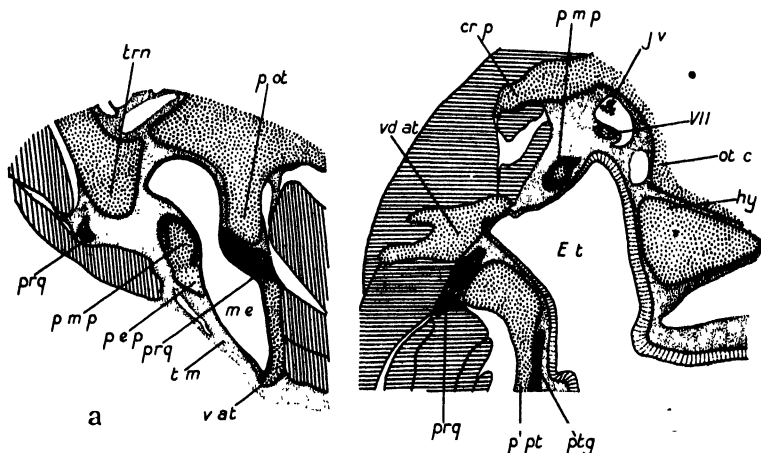
TEXT-FIG. 8 *a, b, c.*



Consecutive transverse sections through the tympanic region of *C. capense* to show its relations with the processes of the pars quadrata. *cr.p.*, crista parotica; *l.a.h.pr.b.*, lower anterior horn of processus basalis; *u.a.h.pr.b.*, upper anterior horn of processus basalis. Other abbreviations as for previous figures.

represent further sections of the auditory apparatus, not differing essentially from Text-fig. 7, *c*, so far as the plectral and annular arrangements are concerned. It will, however, be noticed that the pars externa plectri shifts gradually to a more dorsal position, and in Text-fig. 9, *a*, its dorsal portion shows

TEXT-FIG. 9 *a* AND *b*.



Consecutive transverse sections through the annular region of *C. capense*. *j.v.*, jugular vein; *p.e.p.*, pars externa plectri; *p.m.p.*, pars media plectri; *vd.at.*, ventral and dorsal portions of annulus passing over into each other at the posterior limits of the annulus; *VII*, seventh cranial nerve. Other abbreviations as for previous figures.

perichondral and enchondral ossification, although a marrow cavity is not developed. The section is in fact cut through the transition from pars externa to pars media plectri, which is the only part of the plectral apparatus to ossify in *Anura*. It will be noticed further that the dorsal portion of the annulus has now disappeared from section, although it subsequently reappears. The annulus is in fact not a complete ring as in *Rana*, but is discontinuous dorsally as in *Phrynomerus*. The open, crescentic form of the annulus seems to be quite common in *Anura* and particularly in *Brevicipitid-Engyostomatidae*. Since the region of the pars media plectri is now reached, atten-

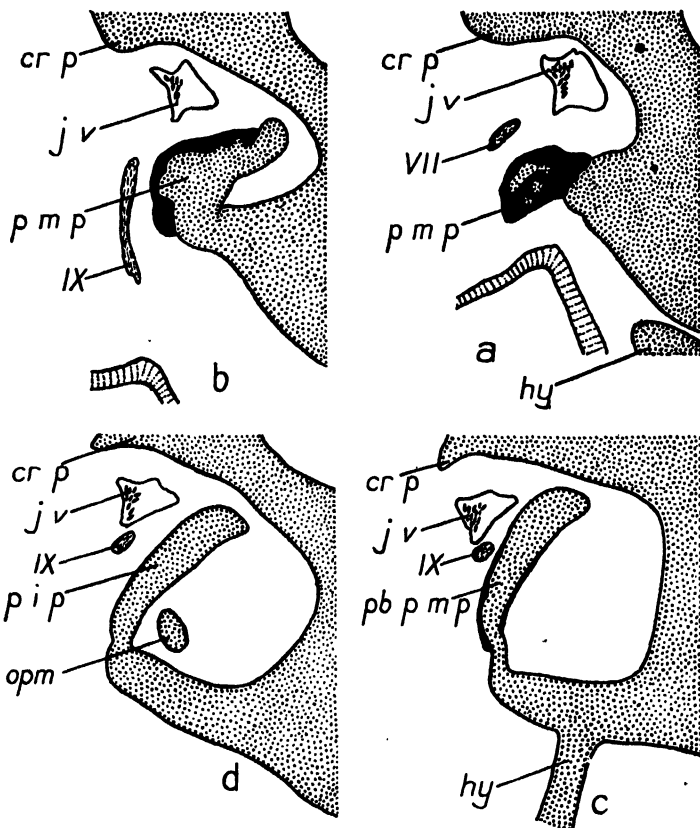
tion should be called to the absence of a pars ascendens plectri effecting a communication between the pars externa plectri and the crista parotica. This cartilage was described by Gaupp for *Rana fusca*, but I have never come across it in South African frogs and toads, so that its universal occurrence in *Anura* should not be assumed. Text-fig. 9, *b*, represents a section through the body of the pars media, which is seen to consist of a perichondral osseous sheath and a central, permanently cartilaginous core. As the pars media approaches the otic capsule, the ossification ceases to be exclusively peripheral and osseous substance may also be seen traversing the cartilage as in Text-fig. 10, *a*. The pars media is overlain by the jugular vein and the hyomandibular branch of the seventh nerve. Text-fig. 10, *b*, represents the point of maximal fusion of the pars media with the otic capsule; it will be noticed that the perichondral ossification is no longer sheath-like, but disappears towards the inner aspect of the pars media, where it is in cartilaginous continuity with the otic capsule. For a short distance the sheath-like pattern is represented again, Text-fig. 10, *c*, and all traces of ossification disappear at the level of the anterior boundary of the operculum (Text-fig. 10, *d*), where the plectrum is represented by the pars interna. The plectral apparatus is therefore a continuous cartilaginous structure, but in the region of the pars media it is perichondrally ossified.

The operculum has the bowl-like shape typical of *Anura* and is fused to the dorsal margin of the fenestra ovalis upon the disappearance of the pars interna plectri. The fossa fenestrae ovalis is large as in *Phrynomerus*. The operculum possesses a well-developed musculus opercularis, which is not, however, attached to a special cartilaginous tuberosity of the operculum.

C. böttgeri has an auditory apparatus which differs quite considerably from that of *C. capense*. The annulus tympanicus is very widely open dorsally, but the plectrum has much the same histological and anatomical structure as in the larger species. The operculum is, however, relatively much larger in the smaller species and in its anterior division it is straightened out, and more watch-glass shaped than bowl shaped. The consequence is that the fossa fenestrae ovalis and the ductus

fenestrae vestibuli both appear to be smaller than in the larger species, although the increase in height compensates for the

TEXT-FIG. 10 *a, b, c, d.*

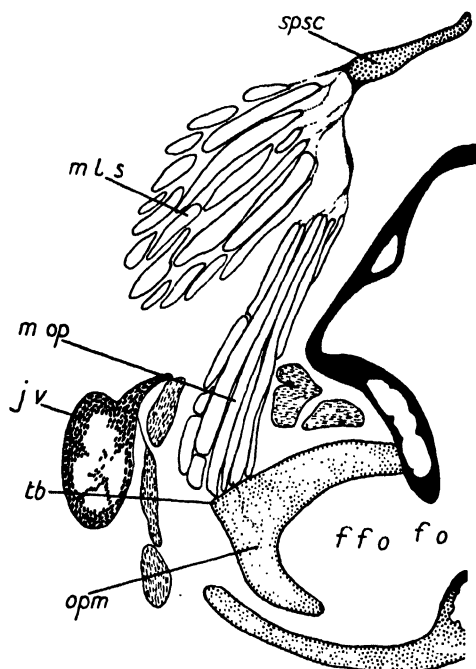


Consecutive transverse sections through the sound-conducting apparatus of *C. capense*. *opm.*, operculum; *pb.p.m.p.*, posterior boundary of the pars media; *p.i.p.*, pars interna plectri; *IX*, ninth cranial nerve. Other abbreviations as for previous figures.

decrease in breadth. Posteriorly the operculum acquires the usual bowl-like shape again (Text-fig. 11). The musculus opercularis is comparatively enormous and joined by means of an

aponeurosis to the musculus levator scapulae. The opercular muscle is attached to the operculum, which develops a special tubercle or tuberosity for this purpose: the tubercle is absent in *C. capense* although the muscle is present. According to

TEXT-FIG. 11.



Transverse section through the opercular region of the ear of *C. böttgeri*. *f.f.o.*, fossa fenestrae ovalis; *f.o.*, fenestra ovalis; *m.l.s.*, musculus levator scapulae; *m.op.*, musculus opercularis; *spsc.*, suprascapula; *tb.*, tubercle for opercular muscle. Other abbreviations as for previous figures.

Versluys (1924) an operculum was evolved as a response to terrestrial life; *C. böttgeri* should therefore be more terrestrial than *C. capense*. Of the latter species I may definitely accentuate the aquatic habits and the same is probably true of *C. böttgeri*.

The hyoid apparatus was imbedded and sectioned with

the skull, but was also dissected out to make sure of the gross anatomy. It was found to be so similar to that of *Rana*, that it was not considered necessary to give a drawing of it. The bay between the manubria is a little shallower than in *Rana*, the processûs alares taper slightly more towards their posterior tips, and the processûs thyroidei are comparatively longer, so that they are still encountered in section towards the posterior limits of the coracosternum. This arrangement is, however, quite secondary and is due to the forward position of the shoulder girdle in the genus. The hyale is fused to the base of the otic capsule behind the processus basalis. The processûs anteriores are absent. The hyoid apparatus in *C. böttgeri* is very similar to that of *C. capense*, but the thyroids are not prolonged posteriorly to such an extent; possibly the pectoral girdle is not so considerably shifted forwards as in the larger species. The hyale, moreover, is not fused with the otic capsule, but merely articulates with it, in a fossa below the lower lip of the fenestra ovalis (Text-fig. 12). It should be further noted that the thyroid processes in both species possess well-developed marrow cavities but end posteriorly in long cartilaginous tips.

The lower jaw lacks all traces of the peculiar modifications encountered in *Phrynomerus*, and agrees in all essential details with that of *Rana*. The mento-mandibular is, however, ossified perichondrally only in the form of a sheath to Meckel's cartilage, whereas in *Rana* enchondral ossification is also initiated. The same conditions prevail in *C. böttgeri* as in *C. capense*.

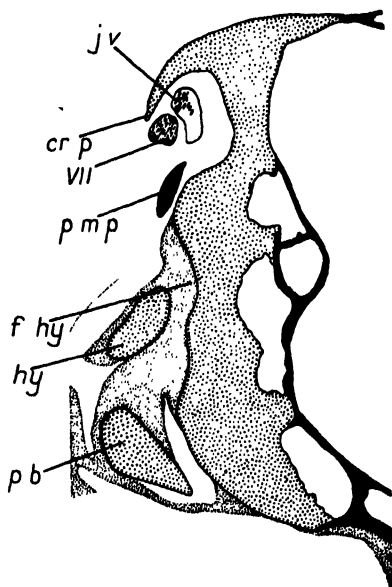
COMPARISON OF THE SKULL OF CACOSTERNUM WITH THAT OF PHRYNOMERUS.

The reasons for investigating the skulls of *Phrynomerus* and *Cacosternum* were (1) to give some new histological details of the Anuran skull in general, (2) to ascertain whether the two Brevicipitid (Engyostomatid) genera are allied, as the absence of a procoracoid might lead one to expect, and (3) to attempt to enumerate some cranial characters by which these two genera are both distinguishable from Ranids; in other words, to attempt to discover some specific Brevicipitid-Engyo-

stomatid cranial characters. It will probably be most convenient to tabulate the evidence under the headings of the various cranial unities.

A. The vestibule of the olfactory organ. The vestibular 'Wülste' described by Gaupp for *Rana* are developed in both

TEXT-FIG. 12.



Transverse section through the otic region of the skull of *C. böttgeri* to show the method of articulation of the hyale. *f.hy.*, fossa in otic capsule for the hyale; *p.b.*, processus basalis. Other abbreviations as for previous figures.

species of *Cacosternum*, and in *Phrynomerus*. The plica obliqua is suspended in *Rana* from the tectum nasi; in the *Brevicipitids* the plica is short and blunt and suspended from the cartilago obliqua.

B. The prenasal cartilages are present in the *Brevicipitids* as in *Rana*.

C. The premaxilla is toothed in *C. böttgeri* and *C. capense* but edentulous in *C. namaquense* (Werner, 1910)

and *Phrynomerus*. [Noble has repeatedly maintained that no great systematic value can be attached to the absence or presence of teeth. The South African *Heleophryne* would, according to Noble, have to be considered as a 'toothed *Bufo*id'].]

D. The maxilla is separated from the outer palatal squame of the premaxilla in *Phrynomerus* only.

E. The eminentia olfactoria is very high in *Phrynomerus*, normal in *Cacosternum* and *Rana*.

F. The vomer in *Phrynomerus* is considerably prolonged into the choana; in *Rana* and *Cacosternum* such dorso-ventral enlargement of the bone does not occur.

G. The palatine in *Phrynomerus* fuses with the vomer to form a vomero-palatine, but in *Rana* and *Cacosternum* the two bones are wide apart.

H. The recessus sacciformis is large in *Rana*, diminished in *Cacosternum*, and purely vestigial in *Phrynomerus*.

I. The nasals in *Phrynomerus* and *Rana* leave little of the anterior portion of the os en ceinture exposed, whereas in the *Cacosternum*-*Anhydrophryne* group the os en ceinture is prolonged anteriorly between the nasals, which are small and laterally placed.

J. *Phrynomerus* has two prechoanal sacs, *Cacosternum* an unpaired one, whereas in *Rana* the structure is absent. The organ in the *Brevicipitids* is to be derived from a prechoanal fusion of the 'Gaumenleisten', and not improbably represents buccal vestiges of Jacobson's organ, which is supposed to be reduced to the recessus medialis in *Anura*.

K. The os en ceinture is paired in *Phrynomerus*, and notched posteriorly in *Cacosternum*, whereas in *Rana* it is girdle-like as its name implies. The lateral trabecular derivatives bounding the fenestra parieto-frontalis are cartilaginous in *Phrynomerus* and *C. böttgeri* but ossified in *C. capense* and *Rana*.

L. The parieto-frontal bones are mere lateral tracts in *Phrynomerus* and *Cacosternum*, whereas in *Rana* they are broad and closely approximated mid-dorsally. Whereas the dorsal cranial roof in the latter genus is therefore bony, it

is formed by thick, densely fibrous connective tissue joining the two parieto-frontals in the two Brevicipitid genera.

M. The optic and pro-otic foramina are bounded anteriorly and posteriorly by bone in *Phrynomerus*. In *Cacosternum* the Ranid type prevails, in which the anterior margin of the optic foramen is not reached by the os en ceinture.

N. The otic capsule in *Phrynomerus* lacks the supra- and sub-cristal ossifications. Weak ossification of the otic capsule is also a feature of *Cacosternum*, particularly of *C. capense*.

O. The transverse taenia of the Ranid cranial roof is absent in both Brevicipitid genera and also in *Arthroleptella*.

P. The occipital region of the adult Anuran skull is remarkably constant in structure. The exoccipitals are joined ventrally by the persistent planum basale. All traces of an intracranial notochord disappear. In *C. capense* the foramen magnum possesses a deep dorsal notch.

Q. The parasphenoid shows no appreciable variation.

R. The paraquadrate is comparatively short in *Phrynomerus* and inclined to fuse with or invade the crista, which is ossified. In *Cacosternum* the crista is cartilaginous, encapsuled, but not invaded by the paraquadrate although the separating connective-tissue lamella disappears.

S. The pterygoid invades and fuses with the ossified periphery of the processus basalis in *Phrynomerus*. This does not take place in *Cacosternum*, in which the separating connective tissue merely disappears. No histological details are available for *Rana*.

T. The quadratomaxillary invades the articular division of the quadrate process in *Rana* (Gaupp), *Arthroleptella*, and *Phrynomerus*. In *Cacosternum* the quadrate cartilage is not ossified, but merely overlain directly by the quadratomaxillary.

U. The two Brevicipitid genera differ from *Rana* in the absence of a processus ascendens plectri and in the incompleteness of the annulus tympanicus. They share these features with *Arthroleptella*. The middle ear and Eustachian tube are present in all the three non-Ranid genera mentioned as well as in *Rana*. The plectrum and operculum are Ranid, except for

the following differences. The operculum is particularly large and shallow in *C. böttgeri*, and develops a relatively large musculus opercularis attached to a special tuberosity. I have not seen an opercular muscle in *Phrynomerus*. The pars externa plectri is enlarged to form an extrapleural imbedded in the mesodermal layer of the tympanic membrane in the two Brevicipitid genera, but not in *Rana*. The pars media plectri lies ventral to the crista in *Phrynomerus*, but in *Cacosternum* is closely applied to the ventral lip of the fenestra ovalis as in *Rana*.

V. The hyalia are fused with the otic region of the skull except in *C. böttgeri*. The Brevicipitid genera lack the anterior processes of the hyoid apparatus, present in *Rana*. The alar processes of *Cacosternum* are like those of *Rana*, but they are enlarged and blade-like in *Phrynomerus*; in the latter genus, as well as in *Cacosternum*, the thyroid processes are met with in the pectoral region, a condition which may be due to the pectoral girdle being shifted forwards. The overlapping is most pronounced in *C. capense*.

W. In the lower jaw the mento-mandibular is exclusively perichondrally ossified in *Cacosternum*. In *Rana* and *Phrynomerus* enchondral ossification also takes place. The latter genus has a remarkably specialized mental region, with backwardly directed diverticula of Meckel's cartilage and a modified gular musculature. These features are absent in *Cacosternum* and *Rana*.

It is always dangerous to base affinities on the study of one particular system of organs only; it is therefore necessary to state explicitly that no final conclusion regarding the mutual relationships of *Phrynomerus* and *Cacosternum* can be arrived at by a comparison of their cephalic skeletons, but that the results embodied in this paper and the previous one on *Phrynomerus* may aid the solution of the problem. The whole question of the validity of the Brevicipitidae as an autonomous family of the Firmisternia, and of the monophyletic origin of the South African Brevicipitid genera, will be fully discussed in a dissertation by one of my students. I shall therefore restrict myself to the cephalic skeleton.

The most important feature which *Phrynomerus* and *Cacosternum* have in common is the extreme reduction of the parieto-frontal bones, and the connective-tissue-like nature of the pretectal cranial roof. Whether the feature is met with in any other Brevicipitids, I cannot say. The otic region of both genera is characterized by the incompleteness of the annulus tympanicus and the enlargement of the pars externa plectri to form an extrapleural cartilage embedded in the tympanic membrane. Moreover, the pars ascendens, effecting a junction of the pars externa plectri and the crista parotica, is not developed. But these features, as well as the absence of a taenia tecti transversalis, are not exclusively Brevicipitid, as they have been proved (de Villiers, 1929) also to occur in the Ranid *Arthroleptella*. The hyoid apparatus lacks the anterior processes in *Phrynomerus* and *Cacosternum*, but again comparative data for other genera are not available, and the processes are also absent in an arciferous toad, *Bufo angusticeps*. The relations of membrane bones like the paraquadrate, quadrato-maxillary and pterygoid, to the cartilages they invest are of great osteogenic interest but are probably not of systematic importance.

Many features of the skull of *Phrynomerus* are indicative of extreme specialization and cannot be used for comparison with *Cacosternum*; such are, e.g., the presence of a vomeropalatine, the absence of a recessus sacciformis, the enlarged eminentia olfactoria and the intrachoanal elongation of the vomer. But these features probably represent individual characteristics of *Phrynomerus* and probably do not represent specifically Brevicipitid modifications. As far as comparative data are available, the skull of *Anura*, with the exception of *Aglossa*, which have become secondarily aquatic, is remarkably constant in structure, and great deviation from the Ranid type should not be expected and has not been recorded in the extant literature on the subject. But it is hoped that the account of the anatomy of the microtomed skulls of *Phrynomerus* and *Cacosternum* may help to revive interest in the osteology of one of the most primitive groups of terrestrial vertebrates; their phylogeny may be obscure, but as

a group they are at any rate end-products of evolution and their genealogy is not obscured by phylogenetic neoteny, as is the case with the Urodeles.

Stellenbosch,
April, 1930.

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Notes on Protodrilus.

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With 11 Text-figures.

THE following paper contains some observations on *Protodrilus flavocapitatus* Uljanin, made chiefly on material from Plymouth. Although Professor U. Pierantoni in his beautiful and valuable monograph on the genus *Protodrilus* (10) added much to our knowledge of these interesting little worms, there remain still many points to be elucidated with regard to their nephridia, genital organs, and mode of reproduction. The work was carried out here, in the Marine Biological Laboratory at Plymouth, and in the Stazione Zoologica at Naples. The worms were studied alive; and also in sections, and in whole and teased preparations of preserved material.

The nephridia.

Nephridia occur in pairs in every segment of the body behind the head in the female and in every such segment in the male excepting the eleventh, which contains the sperm-ducts (the first trunk segment being taken as that which is situated behind the first septum; and this first septum, separating trunk from head, is just posterior to the muscular pharynx). Since Schneider in 1868 (12) first described a Protodrilid ('*Polygordius purpureus*') as hermaphrodite it has generally been held that this condition is characteristic of the whole genus. But, as will appear later on in this paper, there is good reason for believing that this description is founded on a mistaken interpretation, and I shall speak of the worms as male and female.

According to Uljanin's original description of the nephridium of *Protodrilus flavocapitatus* (14), it consists of a long-

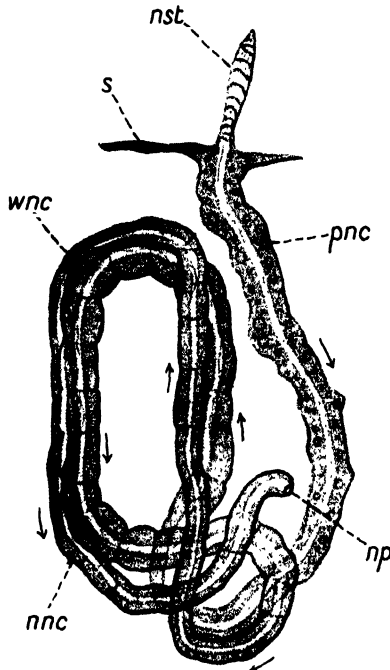
coiled tube formed of a moniliform string of cells pierced by a lumen, without internal opening, and leading to a pore to the exterior situated laterally. Later Hatschek (6) described the nephridium of his *Protodrilus Leuckarti* as formed of a short straight tube bending outwards to a lateral pore, and passing forwards through a septum to open into the coelom of the next segment by a small funnel provided with a flagellum. Salensky, in 1907 (11), gave a more detailed account of the nephridium of *Pr. flavocapitatus*. He says (p. 157): "Ihr vorderes Ende ist ausgezogen, tritt durch das Dissepiment hindurch, erweitert sich trichterförmig und mündet in die vorne liegende Somitenhöhle aus." "Die Trichter sind hinter der Dissepiment gelagert und münden in die Höhle . . . durch die Nephrostomen aus." "Die Nephrostomen sind durch die lippenförmigen hervorragenden Ränder der Trichter begrenzt." "Die Lippe ist innerlich mit starker nach innen gerichteten Wimperhaaren besetzt." Further, the post-septal "Nephrostomkanal" is said to be ciliated and intercellular, and the long glandular canal intracellular.

Pierantoni's description of the same nephridium (10) differs from that of Salensky in that the whole canal is said to consist of a moniliform string of cells with intracellular lumen. A small multicellular open funnel is figured with cilia directed outwards and inwards. The pore is situated in the longitudinal lateral thickening formed where the oblique muscles meet the body-wall.

My own observations differ considerably from those of previous authors. In the first place, the canal of the nephridium of *Pr. flavocapitatus* is much longer and its course more complicated than they suspected. It is difficult to make it out in its entirety; but it appears to be constant in the disposition of, at all events, its main loops (Text-fig. 1). The lumen is ciliated and intracellular throughout. Starting from the funnel is a straight post-septal canal running back along the body-wall below the attachment of the oblique muscles. It is composed of cells of irregular outline, but not separated from each other by a distinct wall. They mostly contain many clear vacuoles. The canal soon bends forwards and changes in character,

becoming moniliform in appearance, being formed of more or less rounded 'drain-pipe' cells set end to end. As shown in Text-figs. 1, 2, and 3, there are two main loops, of which the second and slenderer adheres for the most part closely to the wider first

TEXT-FIG. 1.



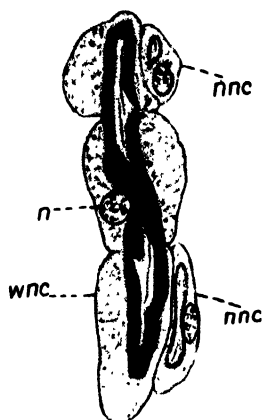
General view of a nephridium, drawn chiefly from the living; enlarged. *nnc*, narrow nephridial canal; *np*, nephridiopore; *nst*, nephridiostome; *pnc*, post-septal canal; *s*, septum; *wnc*, wide nephridial canal. All the figures in this paper are of *Protodrilus flavocapitatus*.

loop and leads finally to the external pore. The inequality in diameter of the two regions is best seen in sections (Text-figs. 2 and 3). These loops, covered by a very thin and perhaps incomplete coelomic epithelium, project into the coelom, and pass between the oblique muscles so as to lie both in the ventrolateral and the centro-dorsal chambers of the segment. The

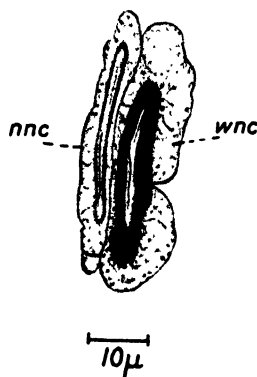
existence of the second more slender loop seems to have escaped the notice of previous observers.

That part of the drain-pipe cells which immediately surrounds the lumen of the thicker loop is formed of dense deeply staining substance, showing slight radiating striations in section, and longitudinal grooves or folds. The lumen itself is often expanded

TEXT-FIG. 2



TEXT-FIG. 3



Sections of parts of nephridial loop showing wide canal, *wnc*, and narrow canal, *nnc*. *n*, nucleus of drain-pipe cell.

between adjacent cells so as almost or quite to interrupt the dense layer. In the more slender region the lumen is bounded by a much thinner layer of similar deeply staining character.

From the above description it will be seen that the nephridial canal of this Protodrilid is of more complicated structure than hitherto supposed, and that it resembles more closely the nephridium of certain small Oligochaeta, such as the Tubificidae, than that of other known genera of Archiannelida or Polychaeta.

The nephridiostome, or 'funnel' of the nephridium, is of peculiar structure, differing from that of any annelid I have studied. It is a delicate somewhat spoon-shaped elongated organ, about 60 μ long, situated near the body-wall on the anterior surface of the septum, and projecting forwards and

upwards into the centro-dorsal chamber of the coelom of the segment in front of that in which lies the canal of the nephridium

TEXT-FIG. 4

TEXT-FIG. 5

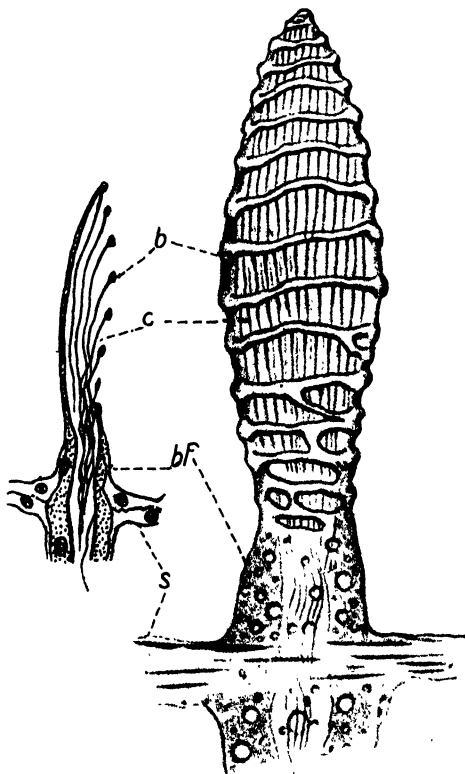


FIG. 4. Diagram of longitudinal section of nephridiostome.

FIG. 5. Much enlarged view of nephridiostome drawn from life. *b*, slender protoplasmic bar across opening of nephridiostome; *bf*, base of nephridiostome; *c*, cilia attached to bar; *s*, septum.

(Text-figs. 1, 4, 5, and 9). The post-septal canal is continued through the septum into the base of the nephridiostome, where its lumen expands towards the opening. Along one side the edge of the opening is continued into a thin protoplasmic lamella convex on its outer side. The aperture on the concave

side of this spoon-like structure is, however, not free but traversed by slender, more or less transverse, protoplasmic bars forming a sort of grid. The bars start from little granular knobs on the rim of the 'spoon' and bear powerful cilia. There are about sixteen such bars, and the cilia attached to them by their stiff base all pass inwards to form a 'flame' beating down into the lumen of the canal (see diagrammatic Text-fig. 4). There is no nucleus in the projecting nephridiostome; a few nuclei are situated in the basal region which pierces the septum.

Since the details of the structure of this nephridiostome can only be made out properly in the living worm, and under a very high power of the microscope (1/12 oil immersion) by focussing through the body wall, it is difficult to make sure of certain details. For instance, although I believe the spaces between the bars are openings from the coelom into the cavity of the 'spoon', it is not impossible that they are in reality closed over by a very delicate membrane. While Salensky seems to have mistaken the whole nephridiostome for one extended lip of an open funnel, Pierantoni does not mention or figure it at all. The few projecting cilia figured by these authors in the region of what they took for the funnel perhaps belong to the general ciliation of the coelomic epithelium described below in the female (p. 814).

The nephridia and genital ducts.

It will be remembered that according to Pierantoni (10) two kinds of nephridia occur in Protodrilids: 'macronephridia' and 'brachynephridia'. The former are provided with a coiled canal with intracellular lumen, are purely excretory in function, occur in both males and females (his 'hermaphrodites'), remain unchanged at sexual maturity, and have nothing to do with the emission of the genital products. Such 'Macronephridia' are found in *Pr. flavocapitatus*, *spongoides*, *Schneideri*, and *oculifer*.

The 'brachynephridia', on the other hand, have a short straight canal with intercellular lumen, and a wide funnel-shaped internal opening. They occur in all the segments of the trunk of both males and females (his 'hermaphrodites'), and are partly of excretory and partly of genital function. 'Brachy-

nephridia' are found in *Pr. purpureus*, *Hatscheki*, *sphaerulatus*, *hypoleucus*, and *Leuckarti*. In mature males certain of these organs become considerably modified, acquire larger funnels, and are in fact converted into specialized sperm-ducts. Pierantoni describes eight pairs of such ducts in segments 8-12 of *Pr. Hatscheki*¹; five pairs in segments 8-11 of *Pr. purpureus*; three pairs in segments 2, 4, 5, of *Pr. hypoleucus*; and two pairs in segments 5 and 6 of *Pr. sphaerulatus*. All these are species provided with 'brachynephridia'; but similar sperm-ducts occur in those species which have 'macronephridia'. For instance *Pr. oculifer* has four pairs in segments 8-11, and one pair only exists in segment 13 (trunk segment 11) of *Pr. flavocapitatus*.

Pierantoni considers that 'macronephridia' and 'brachynephridia' are merely different forms of the same organ. But it seems to me much more likely that, far from being homologous organs, the 'macronephridia' are true nephridia, and the so-called 'brachynephridia' are coelomoducts (1). That the 'macronephridia' of *Pr. flavocapitatus* are true nephridia will not be doubted; the homology of the 'brachynephridia' is more difficult to determine. In favour of the view that they are coelomoducts homologous with those of other Coelomata (1) are the following facts: they serve as genital ducts in certain segments of the male, they have an intercellular lumen, they are provided with a widely open funnel, and the edge of this funnel is in continuity with the coelomic epithelium from which it appears to have been derived (Text-figs. 6, 7, 9). This continuity has already been noticed by Pierantoni (10). It is interesting to note, also, that the ciliated epithelium of the genital funnels of the male has ridges radiating to its periphery closely resembling the ciliated ridges so often found on the coelomostome of *Polychaeta* (Text-figs. 8 and 9).

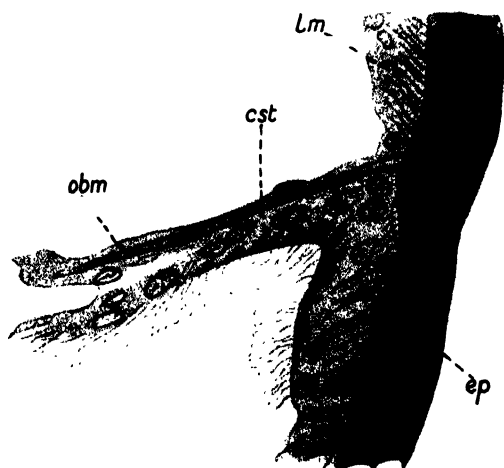
¹ His enumeration of the segments, if I understand him rightly, includes two segments of the head region in addition to those of the trunk enumerated by me.

Professor Pierantoni kindly gave me a slide with sections of *Pr. Hatscheki* showing the sperm-ducts. Some of his observations on this and other species found in Naples I have been able to confirm, and there can be no doubt that his descriptions are in the main correct.

The suggestion is, then, that in those species with true nephridia ('macronephridia'), these organs have persisted in all the trunk segments of the female, and in all the trunk segments of the male excepting those few in which coelomoducts are preserved for the emission of the spermatozoa. The coelomoducts would have disappeared in those segments which have true nephridia. *Pr. flavocapitatus* belongs to this group.

On the other hand, in those species which have no 'macro-

TEXT-FIG. 6.



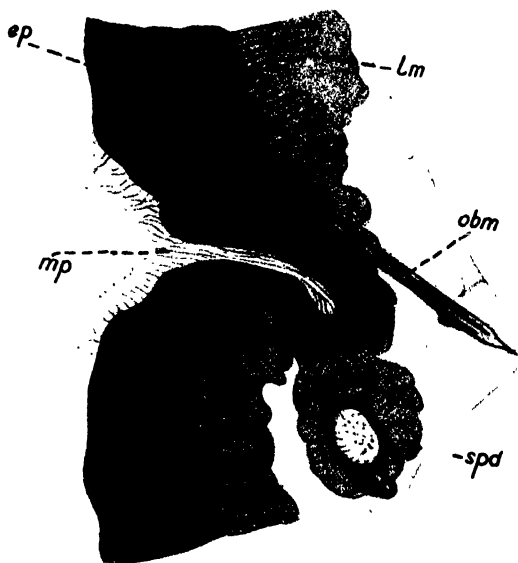
Portion of transverse section of a male, passing through the coelomoduct of the sperm-duct, *cst*. *ep*, epidermis; *lm*, longitudinal muscles; *obm*, oblique muscle.

nephridia', these organs may be supposed to have disappeared in both sexes, while the coelomoducts are preserved in all the trunk segments. They have kept their original function only in those segments of the male in which they develop into sperm-ducts; in the other segments they have become excretory. *Pr. purpureus* belongs to this second group.

The peculiar distribution of nephridia and coelomoducts in different segments seen in the first group of species is closely paralleled by what happens in the smaller *Oligochaeta*, where also the nephridia disappear in the genital segments (1).

Although the interpretation given above seems probable, it must be admitted that the 'brachynephridia' of Protodrilids may possibly be nephromixia, formed by the grafting of the coelomostome, derived from the coelomic epithelium, on to the canal of the nephridium. Such compound nephromixia I have shown to occur in many Polychaeta, where they may function as genital ducts, as combined genital and excretory ducts, or

TEXT-FIG. 7.

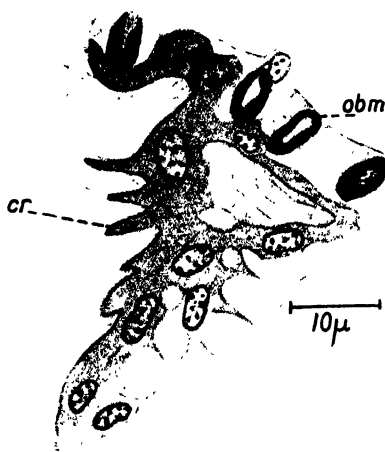


Portion of transverse section of a male, passing through external pore of sperm-duct, *mp*. *spd*, sperm-duct. Other letters and magnification as in previous figure.

even as purely excretory organs (1, 2). But it must be remembered that so far nephromixia have been proved to exist only in Polychaeta, and are unknown in any other group. Although it seems impossible to determine the exact homology of the 'brachynephridia' until their development has been made out, that they are either coelomoducts or nephromixia can scarcely be doubted.

The whole question of the morphological significance of the excretory organs and genital ducts in Archiannelids is still very incompletely understood. Separate true nephridia and genital coelomoducts are known in such forms as *Dinophilus*, *Histriodrilus*, and *Nerilla* (4). The genital funnels of the male *Saccocirrus*, which closely resemble the 'brachynephridia' of Protodrilids, are almost certainly of coelomic

TEXT-FIG. 8.



Section of lip of coelomostome of sperm-duct showing ciliated ridges, *cr.*

origin, but whether the excretory and genital ducts of the female are nephridia or nephromixia is uncertain (3)¹.

¹ When Hempelmann (8) described two pairs of funnels in each fertile segment of the female *Saccocirrus* it seemed that one pair must be nephridiostomes and the other coelomostomes. But I have been unable to confirm his description even in sections of *S. major* which Professor Hempelmann very kindly sent me for inspection. I have been reluctantly driven to the conclusion that the structure he first took to be a slender tube ('nephridial kanal') leading through the ovary to a small funnel is, at all events in the adult, a strand of tissue without lumen—a conclusion with which I believe he himself agrees. It would seem, then, that my original description of *S. papillocerus* holds good (3). Whether the nephridia and ducts of *Saccocirrus* are of the nature of nephromixia could only be determined by studying their development.

The structure and fate of the coelomoducts in annelids are closely related to their method of sexual reproduction. In those forms which emit their genital products in normal fashion they become fully developed, and lead to the exterior either directly (as in Capitellidae, Oligochaeta, and Hirudinea) or indirectly

TEXT-FIG. 9.

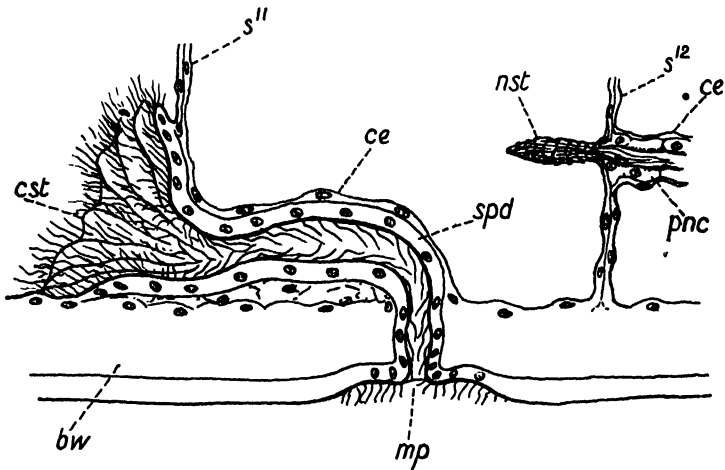


Diagram showing relation of sperm-duct, *spd*, and nephridiostome, *nst*, to eleventh and twelfth septa in a male. *bw*, body-wall; *ce*, coelomic epithelium. Other letters as in previous figures.

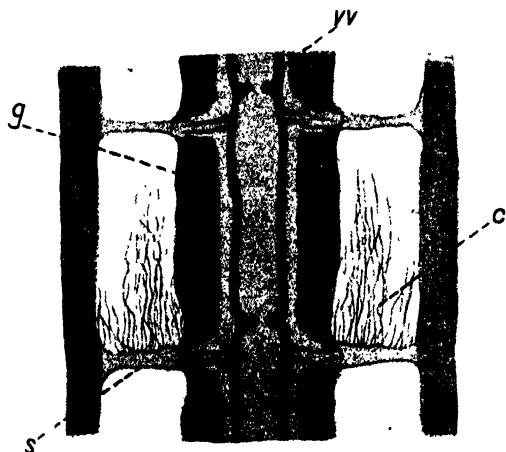
through the nephridial canal (as in Polychaeta with nephromixia). But in those forms where the genital products burst through the body-wall at maturity, or accumulate in posterior segments which drop off and break up, the coelomoducts are apt to become more or less reduced, to cease to open, and even to become modified for other uses (Polygordius, Nereids, Nephthyids [2]). Such also seems to have been their fate in many Protodrilids.

The female Protodrilid sheds its ova by gathering them in posterior segments which become detached and disintegrated; the posterior end then regenerates and the process may be repeated (Pierantoni, 10). Female genital ducts are, therefore,

no longer necessary, and the coelomoducts are either not developed (as in *Pr. flavocapitatus* and other species with true nephridia = 'macronephridia'), or persist only as excretory organs in those species with 'brachynephridia' (possibly nephromixia).

On the other hand, in the male Protodrilid the ripe spermatozoa are normally shed to the exterior. Hence in this sex

TEXT-FIG. 10.



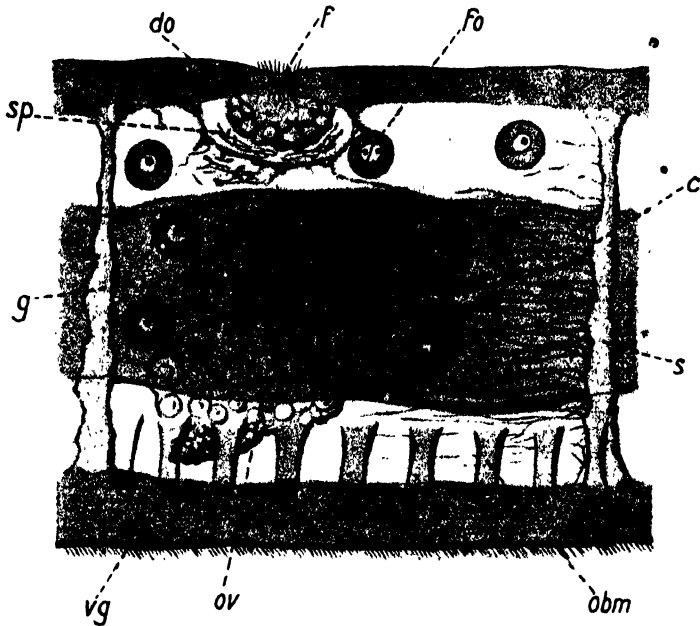
Diagrammatic sketch from the living of a posterior segment of a female to show ciliation of coelomic epithelium. Enlarged ventral view. *c*, cilia; *g*, gut; *s*, septum; *vv*, ventral blood-vessel.

coelomoducts are well developed as sperm-ducts in one or more segments. In these segments the nephridia apparently disappear, much as in the smaller Oligochaeta.

As explained above the female *Pr. flavocapitatus* has only nephridia and no genital ducts; but a fact remains to be described, which seems to have hitherto escaped notice, yet may be of significance in connexion with the reduction of the coelomoducts. On studying living specimens carefully it is seen that in those segments of the trunk which contain ovaries and loose ova (segments from the thirteenth to the pygidium) the coelomic epithelium of the hinder wall is ciliated (Text-figs. 10 and 11). The cilia spring from the coelomic epithelium on the anterior

face of each septum. They are very long and slender, do not beat regularly, but are animated by a wavy motion from base to tip and wave about among the floating ova, which they con-

TEXT-FIG. 11.



Diagrammatic sketch from the living of a posterior segment of a female to show ciliation of coelomic epithelium. Enlarged left-side view. *do*, dorsal glandular organ; *f*, ciliated fossa; *fo*, fertilized ovum; *ov*, ovary; *sp*, ripe spermatozoa; *vg*, ventral ciliated groove. Other letters as in previous figures.

stantly agitate. These cilia are more numerous and longer in the more posterior segments, and one of their functions seems to be to gather the ova towards the posterior end of the female. It is by no means impossible that this ciliated epithelium represents a highly modified remnant of the coelomostomes, which are after all but ciliated funnels developed from the coelomic epithelium.

Sex and reproduction in Protodrilids.

In 1868 Schneider first described *Protodrilus* ('Polygordius') *flavocapitatus* as hermaphrodite (12). After him Ulanin in 1877 (14), and Hatschek in 1880 (6), agreed that the species they described are hermaphrodite. Certain it is that spermatozoa may occur in individuals containing ova. But in this connexion it must be remembered that these authors mistook the salivary glands for ovaries. To Pierantoni (9) belongs the merit of distinguishing the salivary glands in the anterior sterile segments from the true ovaries which occur in pairs in the more posterior segments. Salensky (11) had already concluded that the sexes are separate in *Pr. flavocapitatus*.

Finding undoubted males with a pair of testes in each segment behind the region of the salivary glands, and other individuals with ovaries and also spermatozoa in these segments, Pierantoni concluded that the species is composed of hermaphrodites and complementary males. Moreover, while this author describes normal spermatogenesis ('euspermatogenesis') giving rise to abundant spermatozoa in the male, he states that in the 'hermaphrodite' there is normal ovogenesis giving rise to an abundance of ova, but no normal spermatogenesis. In these 'hermaphrodites' the spermatozoa ("cistospermii") are said to arise in a quite peculiar manner by a process of 'cystospermatogenesis' inside certain cells derived from the coelomic epithelium. He figures sheaves of ripe spermatozoa in these cells, and describes them as developed from a mass of chromatin in the cytoplasm. Self-fertilization is said to occur in the 'hermaphrodite', while the function of the normally produced spermatozoa of the male would appear to be merely to fertilize those ova which may escape to the exterior in an unfertilized condition. From the above brief account it will be gathered that a very extraordinary state of things must exist among the Protodrilids if Pierantoni's interpretation is correct.

My own view is that there is no hermaphroditism in *Protodrilus*—at all events in *Pr. flavocapitatus*, which is the only species I have had the opportunity of studying thoroughly (5). Males and so-called 'hermaphrodites' occur in approxi-

mately equal numbers, and I am convinced that the latter are merely females which, by the time they are full-grown, have generally been inseminated by a male. Some process of copulation no doubt takes place, though it must be confessed that it has not yet been observed. Copulation occurs in several genera of Archiannelida, for instance in *Dinophilus*, *Histiodrilus*, and *Saccocirrus*; but in these cases copulatory adaptations are obvious in the male. No well-defined copulatory organs are found in Protodrilids. In *Pr. flavocapitatus*, however, appearances lead me to believe that the region of the sperm-duct near the male pore is eversible (Text-fig. 7). Definite evidence as to the way in which spermatozoa pass into the female is still wanting, and this is a point for future investigation.

Pierantoni has shown that precocious entrance of the spermatozoon into the oocyte takes place in *Protodrilus* (10), and Hempelmann has described the same process in the closely allied genus *Saccocirrus* (7, 8). The insemination of the female postulated above no doubt occurs early. Shearer showed that in *Dinophilus* copulation may occur even in the cocoon before the young worms have escaped (13).

It is because spermatozoa are often found in individuals possessing ovaries that these worms have been assumed to be hermaphrodite. The bundles of spermatozoa described by Pierantoni in certain coelomic cells I believe to be merely superfluous spermatozoa which have been engulfed in these cells, and not as he supposed stages in 'cystospermatogenesis'. In support of the view that they represent stages in phagocytosis, it may be pointed out that in my experience only ripe spermatozoa are found in the so-called 'hermaphrodite' individuals. It is suggested, then, that *Protodrilus* is always dioecious, and that all individuals with ovaries are of the female sex. The peculiar dorsal organs described by Salensky and Pierantoni in the female of *Pr. flavocapitatus* may possibly be in some way concerned in insemination. These organs are situated in the mid-dorsal line, one in every segment of the fertile region (in every segment of the trunk behind the salivary gland region). The organ consists of a group of large glandular cells opening on a ciliated fossa (Text-fig. 11). Their function has not yet been

made out; but it is to be noticed that spermatozoa are often found, scattered or in bundles, in their neighbourhood, and frequently occur in the space enclosed between the gland-cells and the sac of coelomic epithelium into which they project. Moreover the dorsal organs seem to be better developed in young than in older females in which the ova are very numerous and fertilized. Such organs, however, have not been described in other species.

SUMMARY.

The nephridium of *Protodrilus flavocapitatus* is described in detail. With its long-coiled canal and small projecting nephridiostome it is shown to be more complicated than hitherto supposed.

The sperm-duct of the male has a ridged ciliated coelomostome and represents a coelomoduct or possibly a nephromixium. It is argued that the 'brachynephridia' and sperm-ducts of all Protodrilids are of the same morphological nature.

The fate of the coelomoduct is related to the mode of emission of the genital products. In the female of *Pr. flavocapitatus*, which sheds the ripe ova by dropping off posterior segments, the coelomoducts have been lost in all the segments, and in the male in all the segments excepting the eleventh. Remains of the coelomostomes are perhaps represented by the ciliation of the coelomic epithelium in the genital segments of the female.

It is maintained that *Protodrilus* is dioecious, that the female may be early inseminated by the male, that copulation must take place, and that the dorsal glands are perhaps concerned in the process. Ripe spermatozoa only are found in the female, and the so-called stages in 'cystospermatogenesis' are probably stages in the phagocytosis of superfluous spermatozoa.

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The Structure and Post-Embryonic Development of *Vanessa Urticae* (Lepidoptera).

I. The Larval Alimentary Canal.

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With Plate 14 and 10 Text-figures.

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1. INTRODUCTION.

THIS work was begun as a study in the metamorphosis of Lepidoptera. It very soon became obvious that a necessary pre-requisite was a thorough understanding of the anatomy and growth processes of the larva. This paper is thus a preparatory study. Previous papers dealing with the detailed anatomy of lepidopterous larvae are remarkably few. A complete and

detailed account of the anatomy and development of one species seems to me to have considerable value as a basis for all future studies in the comparative morphology and metamorphosis of Lepidoptera. The present paper describes the structure of the alimentary canal and the changes involved in its growth from the time of hatching to the early part of the last larval stadium.

Acknowledgements. I wish to acknowledge my very great indebtedness to Professor W. Garstang for much kindly criticism and advice. My grateful thanks are also due to the Department of Scientific and Industrial Research for the financial assistance accorded to me during the years 1926-8.

2. METHODS.

The technique employed has already been described (Henson, 1929). Almost all the details of anatomy have been worked out on complete series of sections, aided by dissection in salt solution under the binocular microscope where possible. The three primary divisions of the alimentary canal are taken separately. The fore-gut (stomodaeal), mid-gut (endodermal), and hind-gut (proctodaeal) are described in turn.

3. THE FORE-GUT.

(a) Structure of the Larval Fore-gut.

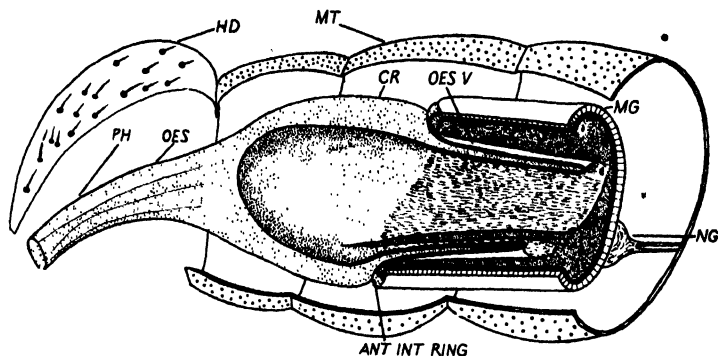
The fore-gut passes backwards from the mouth to the level of the meso-thorax, where it joins the mid-gut. Morphologically it is stomodaeum and is lined throughout with a chitinous intima, continuous with the chitin of the outer surface of the body over the mouth parts. Where it joins the mid-gut it is intucked in such a manner as to produce a double-layered fold projecting into the lumen of the mid-gut (Text-figs. 1 and 2).

Immediately in front of this fold the fore-gut swells out into a kind of crop, and then runs forward as a narrow tube to the mouth. The first half of this narrow tube is pharynx and the second half oesophagus. Between the posterior fold of the valve and the mid-gut epithelium is a thin membranous region composed of very small cells which are frequently seen to undergo mitosis (Text-fig. 2, *ant. int. ring*). These cells belong to the

fore-gut because the intima is continued over them. Bordas (1911) regards these cells as secreting the peritrophic membrane of the mid-gut. Mme Hufnagel (1918) and Deegener (1908) regard them as an anterior imaginal ring comparable to that of the Diptera. In the present paper I have called them 'anterior interstitial ring' for reasons given in the discussion on p. 342.

In *Vanessa urticae* there are thus five separate anatomi-

TEXT-FIG. 1.

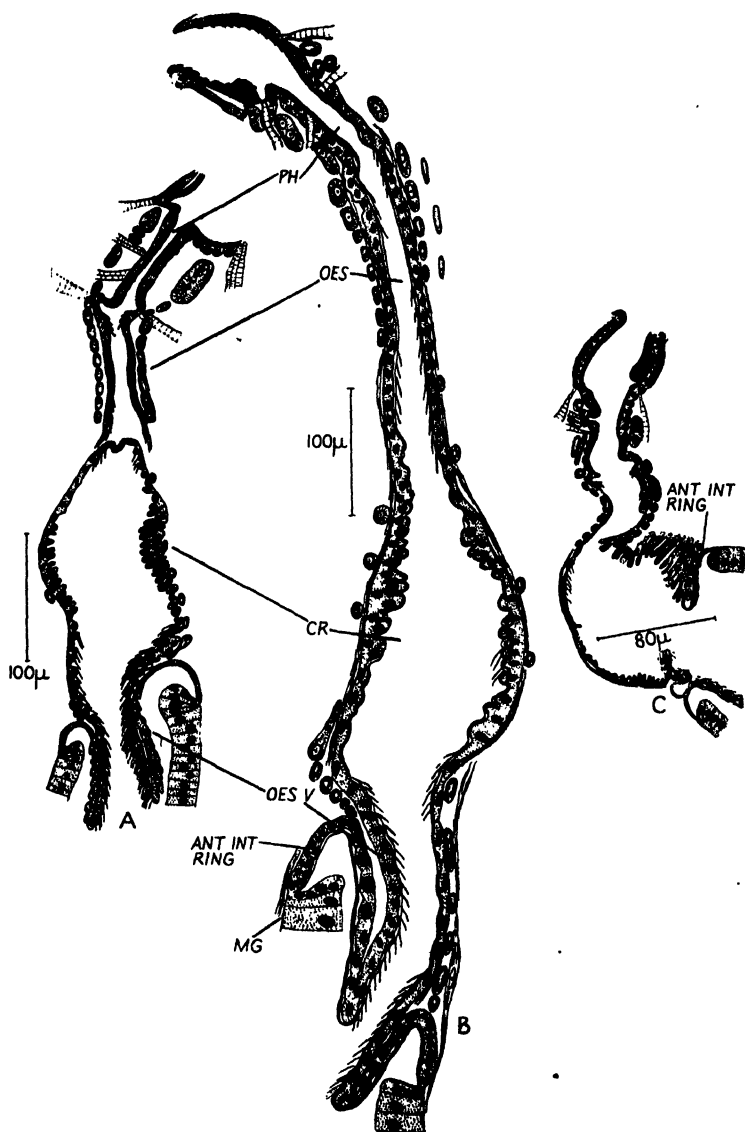


Fore-gut in situ. The crop and oesophageal valve partly cut away. Seen from dorso-lateral side. *ANT. INT. RING*, anterior interstitial ring; *CR.*, crop; *HD.*, head; *MG.*, mid-gut; *MT.*, mesothorax; *N.G.*, nerve ganglion; *OES.*, oesophagus; *OES. V.*, oesophageal valve. *PH.*, pharynx.

cal portions of the fore-gut: pharynx, oesophagus, crop, oesophageal valve, and anterior interstitial ring. The details of the structure of these five regions can now be given.

The Pharynx (Text-fig. 2) is contained wholly within the head capsule and extends from the mouth to the level of the hind-edge of the clypeus. Its posterior limit is marked by the attachment of the posterior dilator muscles (vide below) and by the sudden thickening of the intima at the commencement of the oesophagus. Longitudinal sagittal and transverse sections are shown in figs. 1 and 2, Pl. 14. It will be seen that the epithelium is folded inwards along six sides. This is due to the attachment of the so-called circular muscles along six lines situated dorso-laterally, laterally, and ventro-laterally. Instead

TEXT-FIG. 2.



Long sections of the fore-gut, A and B not quite sagittal. A, early first instar. B, late first instar. C, early second instar. Other lettering as in Text-fig. 1. *Camera lucida*.

of being a cylindrical tube the pharynx is therefore hexagonal and may be said to have a dorsal face, dorso- and ventro-lateral faces, and a ventral face, on each of which lies a layer of muscles (Text-fig. 3). These are therefore best described as transverse bands and not as circular fibres. At the anterior end of the pharynx the ventro-lateral faces are much narrower than the dorso-lateral faces, whilst at the posterior end the reverse is the case.

The epithelium consists of a protoplasmic layer of which the

TEXT-FIG. 3.

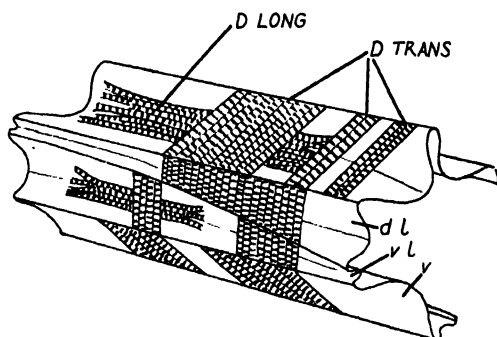


Diagram representing pharynx slit up one side and slightly opened out.

D.LONG., dorsal longitudinal muscle; *D.TRANS.*, dorsal transverse muscles; *d.L.*, dorso-lateral; *v.L.*, ventro-lateral; *v.*, ventral.

surface remote from the intima is raised into blister-like eminences wherever a nucleus occurs. The cells are not separated from one another by complete cell walls; the epithelium is therefore syncytial (fig. 1, Pl. 14). The chitinous intima is prominent but tends to become thinner at the posterior end of the pharynx. It is armed with stout backwardly projecting bristles which probably assist the onward movement of the food in the action of swallowing.

The musculature has features of considerable morphological importance. A comparatively recent paper by Snodgrass (1928) contains a detailed description of the muscular system of the stomodaeum of *Lycophotia margaritosa*.

In describing the muscles in *Vanessa* a slightly different method has been adopted in order deliberately to suggest a

significance additional to that put forward by Snodgrass. Where there seems to be no possibility of confusion, I have adopted his nomenclature and interpretation of the head sclerites.

The muscles of the pharynx fall into three categories, longitudinal muscles, transverse bands, and dilator muscles; the latter pass from the wall of the pharynx to various parts of the head capsule. The longitudinal muscles lie next to the epithelium and are overlain by the transverse fibres in those places where the two co-exist.

Individual fibres may pass over two or more adjacent faces along areas where the bands overlap. It has not been possible to follow the details of this interlacing, but its definite occurrence renders the recognition of distinct muscles rather difficult.

The dilator muscles mentioned above are arranged in two systems, a dorsal and a ventral. The constituent muscles of these systems are all paired right and left (vide Text-fig. 4).

The dorsal system has the following components:

(1) First anterior dorsal dilators (*1st ant. d.*, Text-fig. 4)—running from the dorso-lateral points of the beginning of the pharynx, forwards and upwards to the anterior region of the clypeus. These muscles obviously correspond to the first dorsal dilators of the buccal cavity of Snodgrass.

(2) Second anterior dorsal dilators (*2nd ant. d.*)—arise on the clypeus above the middle and pass forwards on to the dorsal side of the commencement of the pharynx between the members of the previous pair. These again correspond to the second dorsal dilators of the buccal cavity of Snodgrass except that they are medial to the first anterior dorsal and not lateral to them as in *Lycophotia*.

(3) Third anterior dorsal dilators (*3rd ant. d.*)—fixed to the pharynx between the first and second dorsal transverse bands and passing forwards and upwards to the clypeus very close to the submarginal ridge.

(4) Fourth anterior dorsal dilators (*4th ant. d.*)—attached to the pharynx by two strands, one dorsal and one dorso-lateral, immediately behind the second dorsal transverse band, and passing forwards and upwards to the clypeus very close to the previous muscle.

(5) First posterior dorsal dilators (*1st post. d.*)—arise on the clypeus just internally to the epistomal ridge and pass on to the wall of the pharynx dorsally and just behind the third dorsal transverse band.

(6) Second posterior dorsal dilators (*2nd post. d.*)—attached to the pharynx dorso-laterally near the previous pair and passing backwards to the posterior region of the clypeus where the epistomal ridges unite with the frontal ridge.

(7) Third posterior dorsal dilators (*3rd post. d.*)—arise on the cranial wall just laterally to the frons and pass directly inwards to become attached to the pharynx dorso-laterally just behind (6).

(8) Fourth posterior dorsal dilators (*4th post. d.*)—attached dorsally to the posterior end of the pharynx and running upwards to the inner face of the clypeus somewhat anterior to the clypeal attachments of (6). If this muscle corresponds to the similarly-named one of Snodgrass its cranial attachment is not the same as in *Lycophotia*.

The ventral system of dilator muscles does not correspond with the dorsal system (vide Text-fig. 4).

(1) Anterior ventral dilators (*ant. v.*)—attached ventrally to the pharynx in front of the first ventral transverse band and passing backwards to the middle of the transverse bar of the tentorium. These differ from the first ventral dilators of Snodgrass in arising in front of the first transverse muscle and not behind it.

(2) Middle ventral dilators (*mid. v.*)—attached by two fibres, one ventral and one ventro-lateral, to the underside of the pharynx between the two ventral transverse bands, and running backwards to the outer ends of the transverse tentorial bar.

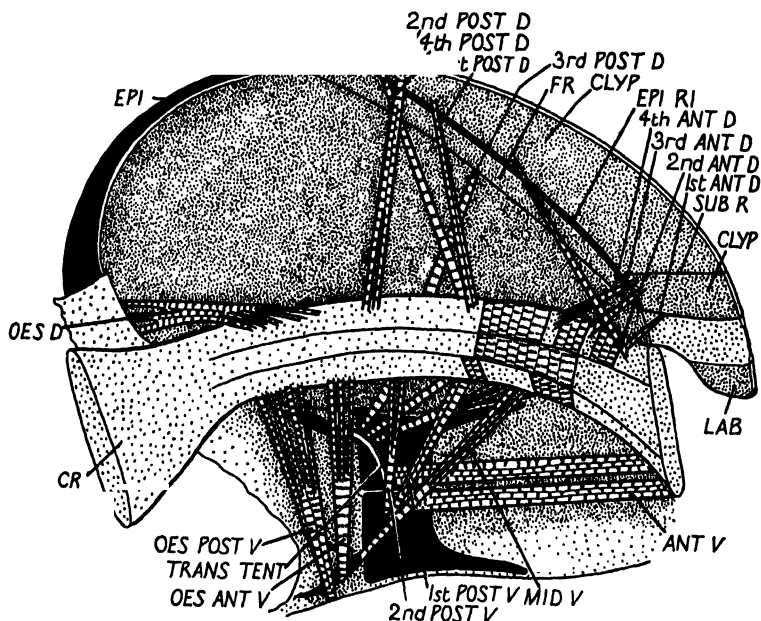
(3) First posterior ventral dilators (*1st post. v.*)—arise ventro-laterally on the pharynx just behind the second ventral transverse band and pass downwards and backwards to the same point as (2).

(4) Second posterior ventral dilators (*2nd post. v.*)—attached by several fibres ventro-laterally to the posterior end of the pharynx and passing downwards to the same point as (2) and (3).

The Oesophagus is the second section of the fore-gut. It extends from the hind end of the pharynx to just beyond the

level of the dorsal margin of the head capsule. Its limits are marked by several features. Its anterior end is defined by the limit of attachment of the pharyngeal dilator muscles and by certain characters of the intima, which becomes thicker and

TEXT-FIG. 4.



Optical section of a larval head to show the dilator muscles of the pharynx and oesophagus. Dorsally only those of the left side are shown. *1st. &c. ANT. D.*, anterior dorsal dilator muscles; *ANT. V.*, anterior ventral dilator muscle; *CLYP.*, clypeus; *CR.*, crop; *EPI.*, epicranium; *EPI. RI.*, epistomal ridge; *FR.*, frons; *LAB.*, labrum; *MID. V.*, middle ventral dilator; *OES. ANT. V.*, anterior ventral dilator of oesophagus; *OES. D.*, dorsal dilator of oesophagus; *OES. POST. V.*, posterior ventral dilator of oesophagus; *1st. &c. POST. D.*, posterior dorsal dilators; *1st. &c. POST. V.*, posterior ventral dilators; *SUB. R.*, submarginal ridge; *TRANS. TENT.*, transverse tentorial bar.

carries rings of stout spines (figs. 1, 8, Pl. 14). At its hind end the longitudinal folding of the epithelium ceases and the transverse puckering of the crop wall becomes evident (Text-fig. 2);

it also begins to widen out into the crop and the strongly developed transverse muscles cease.

The epithelium and intima are histologically similar to those of the pharynx (figs. 2 and 4, Pl. 14). The intima has numerous backwardly directed bristles which are stout enough to be called spines in some areas. As in the case of the pharynx the epithelium is thrown inwards into six longitudinal folds by the attachment of the transverse muscles. The six faces have the same topography as those of the pharynx but are all equally well developed.

The musculature is more regular than that of the pharynx. Thus the transverse bands are strongly and equally developed on all six faces and form an almost complete layer of fibres all round and all along the oesophagus. There are only two longitudinal muscles, a median dorsal and a median ventral. Three dilator muscles are present:

(1) Anterior ventral dilators (Text-figs. 4 and 5, *oes. ant. v.*)—attached to the post-occipital apodemes just laterally to the posterior roots of the tentorium and passing inwards to become attached by a fan of fibres to the ventral and ventro-lateral faces of the anterior part of the oesophagus.

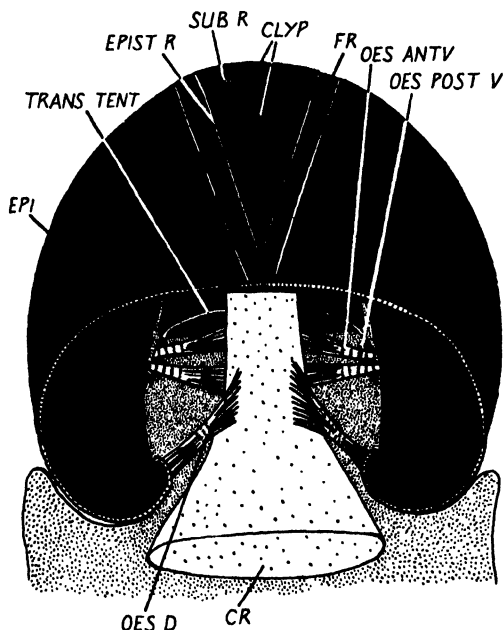
(2) Posterior ventral dilators (Text-figs. 4 and 5, *oes. post. v.*)—attached to the head capsule just laterally to (1) and passing inwards to become attached by a fan of fibres to the ventro-lateral faces of the middle and posterior parts of the oesophagus. Snodgrass describes three muscles of this category, but in *Vanessa* there are only two.

(3) Dorsal dilators (Text-Figs. 4 and 5, *oes. d.*)—attached by a fan of fibres to the dorso-lateral faces of the middle and posterior parts of the oesophagus. Thence they run sharply backwards and become attached by three separate fibres to the hind margin of the head capsule on each side of the dorsal emargination. This muscle obviously corresponds to the dorsal dilators of the oesophagus of Snodgrass. Its triple attachment to the head capsule in *Vanessa* suggests a partial splitting into three muscles, such as are present in *Lycophotia*.

The Crop is a swollen bulb-like portion of the fore-gut contained wholly within the prothorax and mesothorax. The

epithelium (Text-fig. 2) consists of very large flattened cells with no obvious cell limits. The intima is very thin and carries fine bristles on certain areas. The six-sided arrangement such as was found in the pharynx and oesophagus cannot be

TEXT-FIG. 5.



Dorsal view of oesophagus, posterior part of head capsule cut away. *CLYP.*, clypeus; *CR.*, crop; *EPI.*, epicranium; *EPIST. R.*, epistomal ridge; *FR.*, frons; *OES. ANT. V.*, anterior ventral dilator muscle of oesophagus; *OES. D.*, dorsal dilator; *OES. POST. V.*, posterior ventral dilator; *SUB. R.*, submarginal ridge; *TRANS. TENT.*, transverse tentorial bar.

demonstrated in the crop. The mid-gut comes much farther forward on the ventral side than on the dorsal; the crop is thus attached obliquely to the anterior end of the mid-gut.

The musculature is very complicated. In the mid-ventral line is a large longitudinal muscle which is attached to the posterior end of the crop and runs forwards, breaking up into several fibres at the anterior end. On the dorsal and lateral aspects at

the anterior end are many short longitudinal muscles causing the transverse plications seen in this region in the sections (Text-fig. 2). Running round the obliquely placed junction of the crop and oesophageal valve are a number of large, transversely placed muscles forming a kind of sphincter, which probably can completely close the entrance to the mid-gut. Between this sphincter and the anterior region, where the longitudinal fibres occur, are a large number of smaller transverse muscles. In the very restricted region where these fibres encroach on the area occupied by the longitudinal muscles they pass outside them. A number of fine fibres pass over from the mid-gut and become attached to the crop in the region of the posterior sphincter. Where they cross the constituent fibres of this sphincter they are external to them. In *Lycophotia*, Snodgrass (1928) describes the posterior end of the crop as a definite proventriculus. No histological differentiation is present in *Vanessa*, although the musculature suggests a comparison with *Lycophotia*. The presence of the sphincter fibres and the small muscles passing over from the mid-gut are similarities in question.

The Oesophageal Valve is shown in Text-figs. 1 and 2. It is usually described as a cylindrical fold of the fore-gut projecting into the lumen of the mid-gut. In *Vanessa* it is more accurately described as a split cylinder because it is completely absent along the mid-ventral line; the crop epithelium is continuous with the interstitial ring at this point (Text-fig. 2 c). This fact does not seem to have been previously noted in *Lepidoptera* and does not support the view that this fold is valvular in function. The absence of any restraining cords also renders it inefficient as a valve. Perhaps it is best regarded merely as a guide leading the food into the mid-gut.

The epithelial layers will be referred to as the inner or adaxial fold and the outer or abaxial fold (figs. 5 and 6, Pl. 14). The inner layer is more attenuated than the outer and has a much thinner intima. The latter is provided with very numerous bristles on the adaxial fold but is quite smooth on the abaxial. The constituent cells of the folds are not markedly different from those of the crop.

No mesoderm elements of any kind, except a few wandering amoebocytes, have ever been seen between the folds of the valve. It has no musculature.

The Anterior Interstitial Ring is a thin membranous ring composed of very small cells connecting the abaxial fold of the valve with the anterior end of the mid-gut (Text-fig. 2). Up to the end of the third stadium it remains a single sheet. From the period of the third ecdysis to the time of metamorphosis it is folded inwards in its middle part, which thus hangs into the gut lumen like a shelf. Fig. 6, Pl. 14, shows the ring (in section) in its initial form and fig. 7, Pl. 14, in its subsequent condition (cf. Text-fig. 7).

The protoplasm of the ring is not divided into cells but is syncytial and contains a number of small nuclei 2-5 μ in diameter. These have frequently been seen in mitotic phases. The intima over this region is perfectly continuous with that of the abaxial fold of the valve but is very much thinner. This fact is relied upon as proof that the cells are stomodaeal in origin and truly part of the fore-gut.

Perhaps it should be mentioned here that a number of small cells are to be seen on the extreme anterior end of the mid-gut. These are almost indistinguishable in appearance from those of the interstitial ring. In the section on the mid-gut evidence will be given suggesting that they form new mid-gut cells (p. 347).

(b) Larval Development of the Fore-gut.

It is advisable to give definite precision to some of the terms used in the following account. By growth is meant increase in the amount of living tissue in the organ. Increase in size which is not necessarily accompanied by growth is denoted by the term expansion. It has been found that increase in size can take place either by growth or by expansion whilst growth may take place without any marked expansion at all.

The measurements given in this section are merely approximate since only fixed material has been used, and the size of an organ is altered by the state of contraction of its muscles. Thus whilst the measurements serve to bring out the general features they

are not sufficiently exact to determine relative rates of growth, or to be given mathematical precision.

The table below gives a set of data relating to the size of the pharynx at different periods of the life history and the size of its constituent cells as measured by the diameter of the nuclei.

It will be seen that throughout larval life there is a gradual increase in the size of the nuclei of the epithelial cells (Text-fig. 6). From being 3-4 μ in diameter in the newly hatched larva

Growth of Pharynx.

Dimensions in μ .

<i>Phase of Life History.</i>	<i>Dimensions of Pharynx.</i>	<i>Size of Cell Nuclei.</i>	<i>Processes Involved.</i>
Early first instar	90 \times 25	3-4	Expansion and growth.
Late first instar	90 \times 45	4	
Ecdysis period	—	—	Expansion.
Early second instar	—	—	Slight growth and cell enlargement.
Middle second instar	120 \times 65	5-7	
Late second instar	140 \times 60	5-7	
Ecdysis period	—	—	Expansion.
Early third instar	200 \times 100	5-7	Growth, expansion and cell enlargement.
Middle third instar	250 \times 85	10	
Late third instar	320 \times 100	12-15	
Ecdysis period	—	—	Expansion.
Early fourth instar	420 \times 170	12-15	Cell growth.
Middle fourth instar	330 \times 280		
" "	320 \times 200		
" "	300 \times 140	up to 20	
Late fourth instar	400 \times 160	20	
" "	320 \times 200	20	
" "	460 \times 160	20	
Ecdysis period	—	—	Expansion.
Early fifth instar	480 \times 240	20	
" "	720 \times 240	20	
" "	600 \times 200	20	

In the column giving the dimensions of the pharynx the length is given first and then the diameter as measured from the dorso-lateral line of attachment of the transverse muscles to the diametrically opposite ventro-lateral line.

they become 20 μ in the early fifth stadium. This increase in the size of the constituent cells is accompanied by continuous increase in the overall dimensions of the pharynx.

As will be seen later this increase in cell size is characteristic of the whole fore-gut with the exception of the interstitial ring. Only in this latter region have mitotic figures been seen. The ultimate size of the nuclei of the interstitial ring is only 5 μ , a very marked difference from the rest of the fore-gut. It therefore seems justifiable to conclude that in general no cell division occurs in the anterior parts of the fore-gut but only cell enlargement.

Examination of the table shows that the pharynx increases very considerably in size during the period occupied by each ecdysis. This expansion is not accompanied by any increase in the size of the nuclei, thus indicating that no growth occurs in these phases.

In the newly hatched larva the pharyngeal epithelium has the form shown in fig. 8, Pl. 14. The nuclei are quite small (3-4 μ in diameter) and do not cause bulges of the cytoplasm. The intima is very strongly developed. During the first stadium the pharynx doubles its diameter but does not increase in length. The epithelium shows the first signs of development of its normal larval form with a few lateral cell walls (cf. figs. 8a and 8b, Pl. 14). From a general study of all the sections the epithelium does not appear to have suffered attenuation in consequence of the increased size of the pharynx; it follows therefore that addition of its protoplasm must have occurred. The nuclei are no bigger than at the beginning of the first stadium nor are they more sparsely separated.

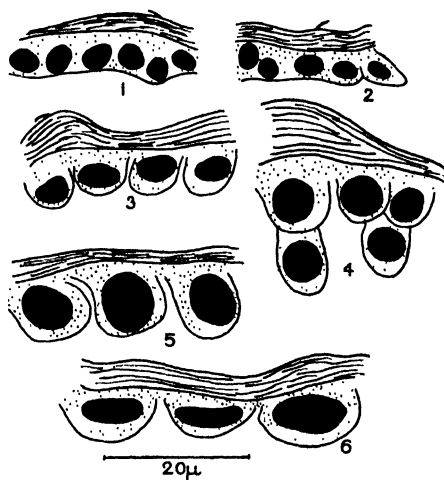
Shortly after the first ecdysis the epithelium has the form shown in Text-fig. 6 (3). It has now definitely developed its lobulated form and the nuclei have increased in diameter. It seems justifiable to conclude that complete differentiation of the pharyngeal epithelium does not take place until shortly after the first ecdysis. The essential features of the differentiation are the development of lateral cell-walls and the development of a tendency to increase the size of the nuclei.

The fact that the nuclei do not increase in size during the first

stadium suggests the possibility of cell division. Close examination of the material, however, has not revealed any mitotic figures.

The only other point of general importance which is revealed by an examination of the table is the relatively great degree of growth which occurs in the third stadium.

TEXT-FIG. 6.



Pharyngeal epithelium at various stages of larval life. 1, early first instar; 2, late first; 3, early second; 4, late second; 5, late third; 6, early fifth. *Cam. luc.*, all to scale.

The Oesophagus is very similar to the pharynx as regards its mechanism of growth and size increase. A comparison of the tables on pages 333 and 336 shows the similarities and differences. It is noteworthy how closely they correspond (in diameter of nuclei) phase for phase throughout most of the larval life. The only real difference is the slight divergence in ultimate size obtained by the nuclei in the early fifth stadium (20μ for the pharynx and 25μ for the oesophagus).

In the case of the Crop the growth phenomena are fairly similar to those of the oesophagus. The data are presented in the table on page 337. It is to be noted that a greater degree of

Growth of Oesophagus.

Dimensions in μ .

<i>Phase of Life History.</i>	<i>Dimensions of Oesophagus.</i>	<i>Size of Cell Nuclei.</i>	<i>Processes Involved.</i>
Early first instar	90 \times 20	3-4	Growth and expansion.
Late first instar	150 \times 40	4	
Ecdysis period	—	—	
Early second instar	—	—	Growth (slight increase in cell size).
Middle second instar	130 \times 65	5	
Late second instar	160 \times 40	6-7	
Ecdysis period	—	—	Expansion.
Early third instar	180 \times 90	6-7	Growth (much increase in cell size) and expansion.
Middle third instar	350 \times 80	8-10	
Late third instar	400 \times 80	12-15	
" "	400 \times 80	12-15	
" "	450 \times 80	12-15	
Ecdysis period	—	—	
Early fourth instar	450 \times 100	12-15	Increase in cell size. Growth and slight expansion.
" "	400 \times 120	12-15	
Late fourth instar	480 \times 120	up to 25	
Ecdysis period	—	—	Expansion.
Early fifth instar	360 \times 250	25	
" "	500 \times 140	25	
Late fifth instar	800 \times 170	25	

In the dimensions of the oesophagus the length is given first and the diameter (as in the case of the pharynx) second.

expansion accompanies the growth in the stadia than in the case of either the pharynx or oesophagus. This may be correlated with the comparative thinness of the intima.

Another point which seems to be of importance is the increase in nuclear diameter during the first stadium. If as seems likely this tendency to increase in size is a characteristic of a differentiated cell as opposed to an embryonic one it indicates that the crop epithelium is in a differentiated condition very shortly after hatching. We have already seen that the pharynx, and oesophagus do not reach the fully differentiated condition until later (i.e. after the first ecdysis). The fact that the nuclei of the crop are bigger (20 μ diameter) than those of the oeso-

phagus (15 μ diameter) at the end of the third stadium also suggests that their enlargement commences at an earlier stage in the life history.

Growth of Crop.

Dimensions in μ

<i>Phase of Life History.</i>	<i>Dimensions of Crop.</i>	<i>Size of Cell Nuclei.</i>	<i>Processes Involved.</i>
Early first instar	270 \times 120	4-5	Growth and expansion.
Late first instar	550 \times 130	5-8	
Ecdysis period	—	—	Expansion.
Early second instar	—	—	Growth and expansion.
Middle second instar	400 \times 280	10	
Late second instar	700 \times 200	15	
Ecdysis period	—	—	
Early third instar	700 \times 200	15	Growth and expansion.
Late third instar	1,000 \times 340	20	
" "	840 \times 480	20	
Ecdysis period	—	—	Expansion.
Early fourth instar	1,200 \times 600	20	Growth and slight expansion.
" "	1,200 \times 700	20-25	
Late fourth instar	very folded.	25	
Ecdysis period	—	—	Expansion.
Early fifth instar	2,200 \times 800	25	
" "	1,700 \times 1,000	25	

The dimensions of the crop given are the length in the mid-dorsal line and the greatest diameter.

The Oesophageal Valve is much the same as the crop as regards growth (vide table, p. 338). Here again the nuclei of the epithelium begin to enlarge immediately after hatching.

Two points seem to be worthy of note. The ultimate size of the nuclei (40 μ diameter) seems at first sight to be much greater than any other part of the fore-gut. However, since they are flattened and sometimes elongated this may not have any real significance.

The other point concerns the intima. The table shows that the rupture of the intima at each ecdysis allows considerable expansion and attenuation of the epithelium to occur. Does the

expansion of the valve during a stadium (particularly the third) mean that the intima is extensible or that it is continually ruptured and repaired? No means of testing this possibility has yet presented itself.

Growth of Oesophageal Valve.

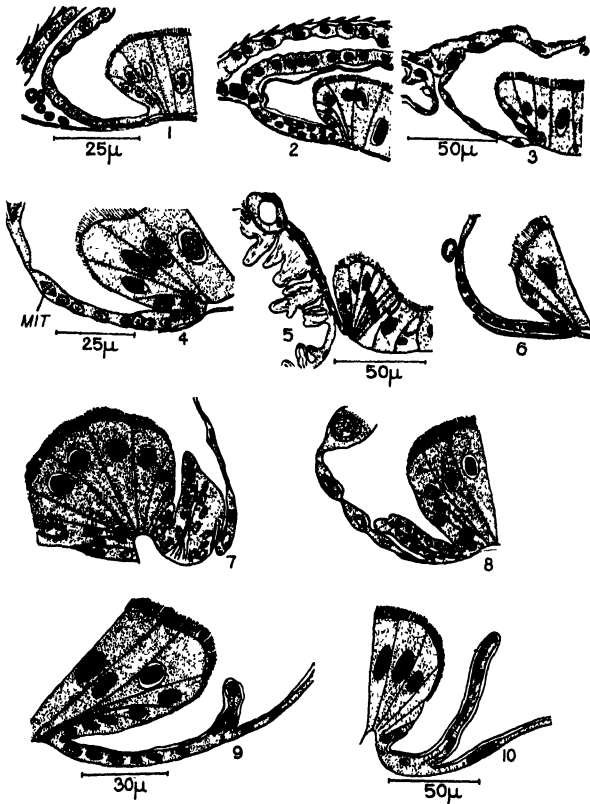
Dimensions in μ .

<i>Phase of Life History.</i>	<i>Length of Valve in mid-dorsal line.</i>	<i>Size of Cell Nuclei.</i>	<i>Processes Involved.</i>
Early first instar	100	3-4	Growth and increase in cell size.
Late first instar	100	6-10	
Ecdysis period	—	—	Expansion and attenuation.
Early second instar	—	—	Growth.
Middle second instar	200	6-10	
Late second instar	180	10-12	
Ecdysis period	—	—	Expansion and attenuation.
Early third instar	270	10-12	Growth and expansion.
Later third instar	280	—	
" "	320	10-12	
Late third instar	380	up to 20	
Very late third instar	400	12-20	
" " "	420	up to 30	
Ecdysis period	—	—	Expansion and attenuation.
Early fourth instar	500	up to 30	Growth and expansion.
" "	600	up to 30	
Very late fourth instar	600	up to 40	
Ecdysis period	—	—	Expansion.
Early fifth instar	720	40	
" "	800	40	

The Anterior Interstitial Ring.—The table dealing with the growth of this region of the fore-gut is given on p. 340. The first and most obvious feature is the fact that the nuclei are never more than $5-6\mu$ in diameter and thereby stand in marked contrast to those of the rest of the fore-gut. In addition the growth is accompanied by mitotic cell division.

Just before an ecdysis many wrinkles and folds appear in the ring and are only straightened out immediately after the casting of the skin (Text-fig. 7). The result of the whole process is a

TEXT-FIG. 7.



Anterior interstitial ring at various stages of larval life. 1, early first instar; 2, late first; 3, early second; 4, late second; 5, early third; 6, middle third; 7, late third; 8, late third; 9, fourth instar; 10, fifth instar; *MIT.*, mitotic figure. *Cam. luc.*

great increase in the diameter of the ring to accommodate itself to the increased diameter of the mid-gut. During the third ecdysis a double fold appears on the inner face of the ring and persists throughout the rest of the larval life.

It will be seen that during each stadium the nuclei tend to increase in size, being about $5\ \mu$ at the end of each of these periods. This occurs in spite of the continuance of mitosis. In periods of ecdysis and in the second stadium when growth is not strongly marked the nuclei tend to decrease in size. There seems to be a kind of balance between rate of growth and rate of mitotic division. Stated in another way there seems to be a tendency towards differentiation which is stopped by the setting in of mitosis. The tendency towards differentiation is shown by the presence of a chitinous intima although only a very thin one.

Growth of Anterior Interstitial Ring.

Dimensions in μ .

<i>Phase of Life History.</i>	<i>Height in mid- dorsal line.</i>	<i>Height in mid- ventral line.</i>	<i>Dia- meter.</i>	<i>Size of cell nuclei.</i>	<i>Processes Involved.</i>
Early first instar	40	40	110	2-3	Growth, mitosis, and expansion.
Late first instar	50	70	180	5	
Ecdysis period	—	—	—	—	Expansion.
Early second instar	—	—	—	—	Growth and mitosis.
Middle second instar	60	60	260	4-3	
Late second instar	60	80	240	3-4	
Ecdysis period	—	—	—	—	Expansion.
Early third instar	80	70	350	4-5	Growth, mitosis, and expansion.
" "	80	70	550	5	
" "	80	80	600	5	
Very late third instar	100	180	450	5-6	
Ecdysis period	—	—	—	—	Expansion.
Early fourth instar	120	180	800	5-3	Growth and mitosis.
" "	100	150	800	4	
Late fourth instar	120	200	700	5-6	
Ecdysis period	—	—	—	—	Expansion.
Early fifth instar	200	160	1,000	5-4	
" "	220	250	1,000	4	

The figures given for the height of the ring indicate what its length¹ would be if pulled out quite straight.

I prefer to regard the ring as a region of unstable differentia-

tion. The cells have retained their embryonic characteristic of readily undergoing mitotic division, and the thinness of the intima seems to indicate the loss of power of chitin production during these phases. I believe that the stomodaeum grows inwards in the embryo by active cell proliferation at its inner end, in fact by a kind of terminal meristem. On this view the anterior interstitial ring represents this persistent embryonic growing point, which does not readily differentiate and remains throughout life in its embryonic condition. It is in fact the growth centre for the formation of the fore-gut although its functioning in this manner is almost restricted to embryonic phases.

(c) General Account of the Larval Fore-gut.

The literature I have consulted on the anatomy of the fore-gut in Lepidoptera is not very consistent with regard to nomenclature. Peterson (1912) speaking of *Protoparce carolina* regards the narrow front end as pharynx and the wide posterior parts as oesophagus. Bordas (1911) dealing with a great variety of forms calls the first narrower part pharynx and the second wider section oesophagus. He then describes the fold as oesophageal valve and the small-celled region between the valve and mid-gut as the generative region of the peritrophic membrane. This latter is a chitinous-like tube passing backwards over the surface of the mid-gut epithelium. This view requires some considerable analysis and will be examined when dealing with the peritrophic membrane (p. 344). In the anatomical part of this paper, referring to the Arctiidae, he suggests that the posterior part of the oesophagus corresponds to the 'jabot' of the adult, thus almost recognizing the presence of a definite crop. Snodgrass (1928) recognizes pharynx, oesophagus, and crop in *Lycophotia margaritosa*.

Mme Hufnagel (1918) writing on *Hyponomeuta padella* divides the fore-gut into buccal cavity (the region round the mouth parts), pharynx, oesophagus, oesophageal valve, and anterior imaginal ring. This latter is the small-celled region between the valve and the mid-gut and thus corresponds to the generative region of the peritrophic membrane of Bordas.

Deegener (1908) adopts the same terminology for *Malacosoma castrensis*.

The anterior imaginal ring of these authors is so named because of its behaviour during metamorphosis. It is regarded as a persistent embryonic region whose function is to form certain parts of the imaginal fore-gut. This viewpoint is put forward most convincingly by Pérez (1910) in dealing with the metamorphosis of *Calliphora*. In the metamorphosis of the *Lepidoptera* it does not play nearly so great a part in the re-formation of the imaginal gut as it does in *Diptera*.

It is difficult to reconcile Bordas's view of its function (as a mechanism for the secretion of the peritrophic membrane) with the view which regards it as an undifferentiated embryonic region or imaginal ring. In the present paper it has been called anterior interstitial ring, because it is present in essentially similar form in both larva and imago. It plays so little part in the metamorphosis that it can scarcely be regarded as an embryonic region set aside for the production of imaginal tissues.

Our ideas with regard to metamorphosis have their foundations in studies upon dipterous subjects and have thence been applied to the other orders. Surely this is the wrong way round. The nature of metamorphic changes should be analysed on a less specialized order and then applied to *Diptera*. On the view put forward in this paper, the interstitial ring is the persistently embryonic terminal part of the stomodaeum and may be used to enable great change of form to take place in insects which have specialized in metamorphosis. It need not be used in this way and there is nothing against its occurrence in heterometabolous forms.

Reviewing the growth of the fore-gut as a whole there are one or two points to be noted. In the newly hatched larva the epithelium is very similar in all parts; the nuclei are all 3-5 μ in diameter and very similar in appearance. During the first stadium marked differentiation occurs in the crop and valve, less marked differentiation in the pharynx and oesophagus, whilst the interstitial ring retains its early features and power of mitosis, i.e. it retains its embryonic character. In marked contrast to the crop and valve the pharynx and oesophagus

show no increase in the size of their nuclei until after the first ecdysis. This suggests the possibility of cell division in these regions during the first stadium (vide pp. 334-5). Should mitosis be observed it would mean that this region tends to retain its embryonic condition longer than the more posterior parts of the fore-gut, and would suggest the possibility of an incipient 'imaginal' ring comparable to the one described in a corresponding position in the blow-fly larvae by Pérez (1910).

In all parts of the fore-gut, except the interstitial ring, growth in larval phases is accompanied by increase in cell size and not by increase in cell numbers. Ecdysis periods are characterized by expansion of the various regions; the evidence suggests that growth ceases during these times.

4. THE MID-GUT.

(a) Structure of the Larval Mid-gut.

The mid-gut has the form of a long tube of uniform diameter extending from the level of the mesothorax to about the middle of the sixth or seventh abdominal segment. In early larval phases it is quite straight, but in older larvae it seems to be too long for the space available and becomes irregularly bent. It is held in place mainly by its tracheal attachments. Its musculature consists of a network of fine fibres, the circular ones being internal to the longitudinal. It is interesting to note that in *Protoparce* (Peterson, 1912) the longitudinal muscles are in six bands. This arrangement of the gut muscles in sixes is well seen in the fore and hind-guts of *Vanessa*, but it cannot be demonstrated in the mid-gut.

The epithelium has a definite basement membrane on which are found three kinds of cells. These are (1) ordinary columnar epithelial cells, (2) goblet cells, and (3) interstitial cells. In a previous paper (Henson, 1929) it has been shown that these latter cells are embryonic rudiments which continually undergo mitotic division, and during periods of ecdysis add new columnar and goblet cells to the epithelium.

The columnar cells have a number of fine, thread-like processes on their lumen faces collectively known as the 'striated

hem or border'. The goblet cells have exactly similar threads lining the cavity of the goblet. These two kinds of cells are regarded by me as closely allied morphologically. Their only essential difference seems to be that in the goblet cell the fibril-producing surface is so great that it becomes tucked inwards, whilst in the columnar cell it remains superficial. This renders the relationships of these two kinds of cells *inter se*, and with those of the mid-gut of insects which do not possess goblet cells, fairly clear.

The Striated Border has formed the subject of much fruitless discussion. The prevailing opinion seems to be that it is composed of cilium-like processes. Vignon's observation (1901) of having seen them in vibration seems to have been due to the presence of intestinal spirochaetes (Léger, 1902). Its presence inside the goblet cells suggests that it may be some kind of secretion and be produced by ejection from the goblet cells. This is not so, however, because it is present as a low fringe on the anterior terminal cells of the mid-gut where no goblet cells exist, and it is also present in the newly hatched larva when goblet cells are only just differentiating. Further, as I have already shown (*loc. cit.*, pp. 90 and 91), the border increases in height correspondingly with the growth of the cells which possess it. These facts seem to make it impossible to regard the border as anything more than a morphological feature of the cells carrying it. Its presence may be correlated with processes of secretion or absorption.

Peritrophic Membrane.

In view of the recent appearance of a paper by Mr. V. B. Wigglesworth on this subject, the earlier work need only be considered very briefly.

Two views have been advanced with regard to the formation of this membrane in Lepidoptera. Vignon (1901) writing of *Bombyx* regards it as formed by delamination from the entire surface of the mid-gut. Bordas (1911) regards it as being formed by the small cells in the angle where the mid-gut joins the fore-gut; that is to say by the cells which I have called 'anterior interstitial ring'.

The papers dealing with the formation of this membrane in Diptera are much more precise and convincing than those dealing with other orders. Van Gehuchten (1890) showed that in *Ptychoptera contaminata* it is formed by secretion from a special band of cells at the anterior end of the mid-gut. It is important to notice that these cells are definitely mid-gut. Vignon (1901) shows a very similar state of affairs in *Chironomus*; again the cells concerned definitely belong to the mid-gut. Close examination of Wigglesworth's figures in *Anopheles* and *Culex* also indicates the same thing. In his paper on 'Digestion in the Tsetse-fly' the cells concerned show a striated border and are therefore genetically mid-gut.

To return to Bordas's paper—the cells he figures as secreting the peritrophic membrane are undoubtedly the anterior interstitial ring and therefore belong to the fore-gut. Such an interpretation seems to me to be fundamentally at variance with the results obtained on Diptera. I have already shown that the anterior interstitial ring has a chitinous intima continuous with that of the oesophageal valve. It is difficult to see how it could have this and also secrete a peritrophic membrane. Further, it is not easy to reconcile the view which regards it as a sort of gland secreting this membrane with the view which regards it as an embryonic region or imaginal ring. Lastly, in examining dozens of larvae I have never seen appearances such as are given in Bordas's fig. 16. May I be allowed to suggest that what Bordas really saw was the reflexed lining of the fore-gut in a larva which had just moulted?

In *Vanessa urticae* I believe that the peritrophic membrane arises by secretion from a considerable area of the anterior end of the mid-gut. It may also be reinforced by secretion from the cells of the mid-gut along its whole length. In young larvae it is present but is not easily dissected out. In older larvae it is readily seen as a delicate colourless membrane investing the food in the mid-gut. Examined in surface view it appears homogeneous and without markings of any kind. In transverse sections it appears to consist of several layers (fig. 10, Pl. 14). In regions posterior to the oesophageal valve, the movements of the gut contents will tend to free it from the underlying cells

and draw it out backwards. Where it is covered by the valve it will be preserved from this action and will stick more closely to the cells producing it. This is readily observed if the gut is cut open and the peritrophic membrane pulled out; it usually brings out with it parts of the epithelium from the anterior region. Further evidence supporting the view that the membrane arises as described above can be obtained from a longitudinal section of a newly hatched larva (fig. 11, Pl. 14). In this phase only parts of the egg-shell are present in the gut and the relationships of the membrane and the epithelium are not seriously disturbed. The section shows that in spite of shrinkage due to the technique, the membrane follows the contour of the epithelium along the whole anterior region covered by the valve.

This account agrees in some respects with that of Pavlovsky and Zarin (1922) for the honey-bee, and also apparently with that of Voinov¹ (1898) for *Aeschna*. Wigglesworth (1930) overcomes the difficulty of reconciling the work on Diptera with these two papers by assuming that the membrane is formed from an anterior ring and then reinforced by additions from mid-gut cells farther back. He suggests that these additions are possibly not chitinous. In speaking of Pavlovsky and Zarin's work he says 'Moreover, the membrane gives the reactions of chitin, a fact which does not support the idea that it is shed off from the entire mid-gut'. However, his own beautifully precise work on the tsetse-fly and on *Anopheles* and *Culex* shows quite clearly that the anterior mid-gut cells do produce the chitinous membrane, and if the anterior cells can produce chitin, why not the whole mid-gut in more generalized forms?

From consideration of the whole subject it does not seem to me to be very likely that the small cells in the angle where mid-gut joins fore-gut are really involved in the secretion of the membrane. Balbiani (1890) describes it in *Cryptops* as being split off from the entire surface of the mid-gut. Here there is no oesophageal invagination and no 'press-mechanism'. In *Lepidoptera*, *Hymenoptera*, and *Odonata* I believe that the membrane is secreted by a considerable extent of the anterior part of the mid-gut and possibly reinforced by secretion from

¹ Cited by Wigglesworth 1930.

behind. In Orthoptera, Dermaptera, and Diptera the specialization has been carried still farther and the secretion is restricted to specialized anterior parts of the mid-gut, which are sometimes its extreme anterior end. In all cases it is a product of the mid-gut only.

(b) Development of the Larval Mid-gut.

Most of the facts with regard to this subject have already been published (Henson, 1929). However, a few generalizations may be given.

Just as the anterior interstitial ring is the growth centre for the formation of the fore-gut, and the posterior interstitial ring the centre for the hind-gut, so are the interstitial cells the growth centres for the mid-gut. The activity of the interstitial rings is confined chiefly to embryonic phases, but the mid-gut interstitial cells continue their functions into larval and post-larval stages. It is interesting to observe that although these cells are embryonic, the kinds of cells into which they can differentiate are fixed. It seems probable to me that the initial separation of the germ layers is followed by their subdivision into centres for the production of the separate tissues.

Just as in the case of the fore-gut, the differentiated cells (columnar and goblet cells) increase in size as they become older, whilst the interstitial cells, like those of the interstitial rings, divide by mitosis and remain small. Their size seems to be controlled by a balance between rate of growth and rate of division.

No mention has yet been made of the structure of the two ends of the mid-gut. At both anterior and posterior ends the cells gradually become smaller and smaller until they are almost indistinguishable from the cells of the anterior and posterior interstitial rings (figs. 6, 7, 9, Pl. 14, and Text-fig. 7).

I believe that these terminal small cells continually divide and grow, thus adding new cells to the mid-gut just as do the interstitial nests. The reasons for adopting this view are as follows: The mid-gut continually increases in diameter at its two ends; this could only be accommodated by increase in cell size or cell numbers. If the former process occurred the end cells of the mid-gut would continually increase in size as the larva aged.

However, the transition from small cells adjacent to the interstitial ring to normal mid-gut cells is always gradual. Therefore presumably new cells are added. The striated border begins on some of the intermediate cells as a very low fringe which gradually becomes higher as the normal mid-gut cells are reached. We have already seen that it is characteristic of a half-grown cell to have a low fringe (*loc. cit.*, pp. 90 and 91), and therefore these end cells are in process of growth. These terminal interstitial cells seem never to produce goblet cells.

The changes occurring in the fifth stadium were described in my previous paper (1929) in terms of the work published on the metamorphosis of *Lepidoptera* by Deegener (1910) and Mme Hufnagel (1918). Work on the metamorphosis is now proceeding, but I should like to give a new interpretation of fig. 13, Pl. 5 (*loc. cit.*). Below the imaginal cells are to be seen some very tiny cells. I believe that the larval interstitial cells mostly differentiate into imaginal cells during the fifth stadium, but some remain behind as the very small cells mentioned above. The imaginal cells are produced from the mid-gut growth centres in exactly the same way as the differentiated cells of the larval mid-gut. There is no separation of larval and imaginal tissues in the egg, but the growth processes of metamorphosis are specializations of those of the larva. Does this view clear up the relationships between the regenerative cells of the mid-gut in *Heterometabola* and the 'imaginal' cells of the *Holometabola*?

5. THE HIND-GUT.

(a) Structure of the Larval Hind-gut.

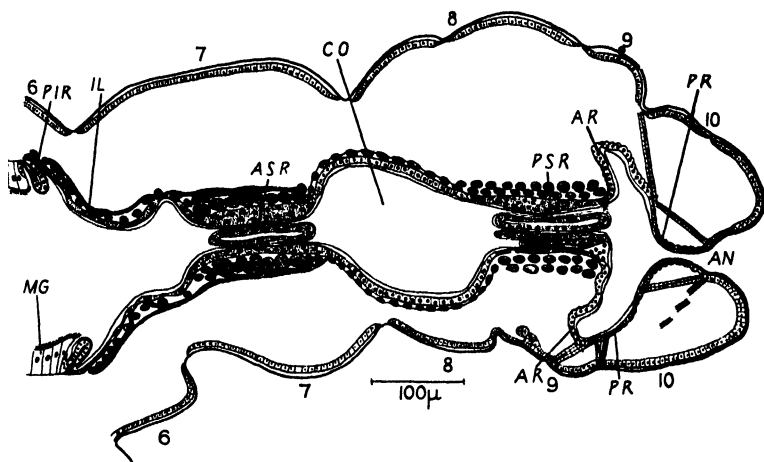
The hind-gut is an inclusive term for that part of the alimentary canal extending from the posterior end of the mid-gut to the anus. It is a morphological entity derived from the proctodaeum of the embryo and is lined throughout with a chitinous intima.

Within the order *Lepidoptera* there seems to be very considerable variation as regards the number of definite regions into which it can be divided (Bordas, 1911). This variation

tends to create difficulties of nomenclature, and that suggested by Imms (1925) has been adopted in the following account.

The terminal region of the proctodaeum adjoining the mid-gut has a ring of embryonic cells similar to that found in a comparable position in the stomodaeum. This has been called the 'posterior interstitial ring', just as that of the stomodaeum was

TEXT-FIG. 8.



Longitudinal sagittal section of the posterior end of the body to show the larval hind-gut. AN., anus; A.R., anterior rectum; A.S.R., anterior sphincter region; CO., colon; IL., ileum; MG., mid-gut; P.I.R., posterior interstitial ring; P.R., posterior rectum; P.S.R., posterior sphincter region. *Cam. luc.*

called 'anterior interstitial ring'. In *Vanessa urticae* the rest of the hind-gut seems to be subdivided to the maximum degree found in *Lepidoptera*. Seven distinct regions have been recognized: (1) posterior interstitial ring, (2) ileum, (3) anterior sphincter region, (4) colon, (5) posterior sphincter region, (6) anterior rectum, (7) posterior rectum (Text-fig. 8). The posterior interstitial ring corresponds to the imaginal ring of previous authors. The rectal cavities have not been separated hitherto from one another, but their histological and anatomical characters are quite distinct.

The musculature of the hind-gut is much more strongly developed than that of the fore-gut.

As in the case of the pharynx and oesophagus the transverse muscles are attached along six lines placed dorso-laterally, laterally, and ventro-laterally. The six bands of transverse muscles so defined, however, are not rigorously separable, since individual muscle fibres may form parts of two or more adjacent bands, and thus bring about a partial continuity between them. This kind of interweaving was noted in the description of the pharynx but in order to avoid complication of descriptions was not stressed. In some parts of the hind-gut, dilator muscles, six in number, pass to the body wall from the above-mentioned six lines of muscle attachment. This six-radiate arrangement may be of considerable significance in insect development, particularly as six is commonly accepted as the primitive number of Malpighian tubules.

The Posterior Interstitial Ring (figs. 9a and 9b, Pl. 14) has the form of a double fold projecting into the gut-lumen. Between the layers of this fold circular muscle fibres are to be found. Longitudinal muscles pass over from the mid-gut and become attached to the posterior fold where it joins the ileum.

Each fold normally consists of a layer of protoplasm with a single row of nuclei. Cell walls are only occasionally observed. The nuclei of the anterior fold are usually more numerous and smaller than those of the posterior fold.

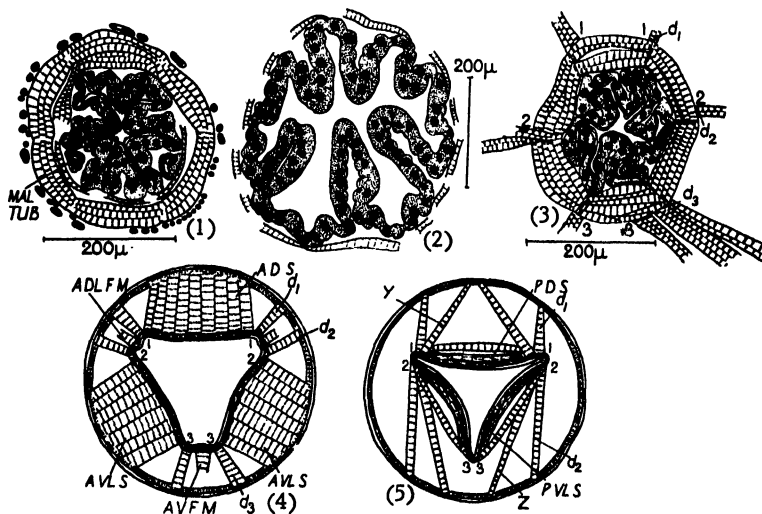
A chitinous intima is present on both layers, thus proving that these cells are morphologically part of the hind-gut. The intima on the posterior layer has numerous transverse rows of fine bristles such as were found on various regions of the fore-gut.

The ring is present in exactly this form in the gut of the imago. For this reason it seems scarcely logical to call it the imaginal ring, especially as it contributes very little to the re-formation of the imaginal hind-gut during the metamorphosis. This fact has the same significance as the similar fact brought forward in connexion with the anterior interstitial ring.

The Ileum is a short, wide, funnel-like tube leading back from the posterior interstitial ring to the anterior sphincter

region (Text-fig. 8). The epithelium consists of large cells and the intima is quite smooth. In the musculature only transverse fibres and an outer system of longitudinal muscles are present. Some of the latter run along its whole length, whilst others only cover its posterior half.

TEXT-FIG. 9.



Sections and diagrams of parts of the hind-gut. (1) Transverse section of anterior sphincter region (*cam. luc.*). (2) Transverse section of colon (*cam. luc.*). (3) Transverse section of posterior sphincter region (*cam. luc.*). (4) Diagram of anterior muscles of the posterior rectum. (5) Diagram of posterior muscles of the posterior rectum. *A.DL.F.M.*, anterior dorso-lateral face muscles; *A.D.S.*, anterior dorsal muscle sheets; *A.V.F.M.*, anterior ventral face muscle; *A.VL.S.*, anterior ventro-lateral muscle sheets; *d₁*, *d₂*, *d₃*, dorso-lateral, lateral, and ventro-lateral dilator muscles; *MAL. TUB.*, common duet of Malpighian tubules; *P.D.S.*, posterior dorsal muscle sheet; *P.VL.S.*, posterior ventro-lateral muscle sheet; *Y, Z*, muscles; 1, 2, 3, similar topographical points.

The Anterior Sphincter Region (Text-figs. 8 and 9 (1)) is a short tube-like portion of the hind-gut immediately behind the ileum. The epithelium has no special peculiarities, but the intima is characterized by having a row of stout bristles anteriorly and by being clothed with very numerous fine ones

behind. The only muscles present are the transverse and an outer series of longitudinal fibres. The transverse muscles are very strongly developed. Some of the longitudinal muscles pass from the anterior to the posterior parts of the region, whilst others pass forward from its middle on to the ileum.

The Colon (Text-figs. 8 and 9 (2)) is a globular or ovoidal chamber between the anterior and posterior sphincter regions. The epithelium is composed of large cells and the intima is quite smooth and devoid of bristles. The muscle system is much reduced, only the transverse muscles being present. These consist of a number of equally spaced fibres often seen to be connected together by a thin membrane.

The Posterior Sphincter Region (Text-figs. 8 and 9 (3)) is characterized by its very strongly developed transverse muscles. There are no longitudinal fibres. Six dilator muscles are present (Text-fig. 9 (3)). The two dorso-laterals (d_1) are more anterior than the lateral (d_2) and ventro-lateral pairs (d_3). All six pass outwards and become attached to the body-wall between segments eight and nine.

The epithelium has no special peculiarities and the intima is quite smooth.

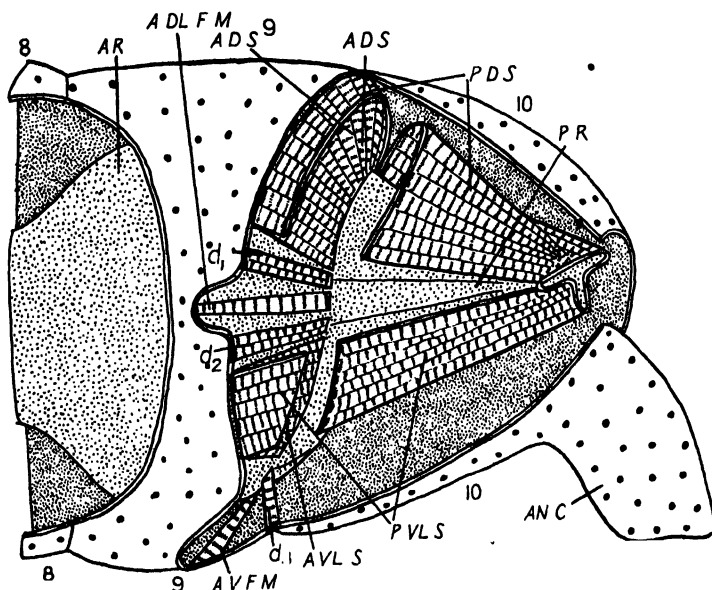
The Rectum has always been described as the globular chamber at the posterior end of the hind-gut. In reality it is two definite structures of quite different form. These will be referred to as anterior rectum and posterior rectum. The line of junction of these two parts is somewhat obliquely placed, coming farther forward ventrally than dorsally (Text-fig. 8 and fig. 12, Pl. 14).

The Anterior Rectum (Text-figs. 8 and 10, and fig. 12, Pl. 14), forms the first half of the globular chamber. Its epithelium is composed of very large cells and its intima is quite smooth. It has no musculature whatever. Its greatest peculiarity is its very close association with the terminal convolutions of the Malpighian tubules. These latter lie in six groups closely investing the epithelium (fig. 12, Pl. 14).

The Posterior Rectum (Text-figs. 8 and 10, and fig. 12, Pl. 14) joins up to the anus, which seems to be formed by a secondary intucking of hypodermis as distinct from proctodaeum. The whole of the posterior rectum is funnel-shaped and

three-sided in cross section. Comparison of Text-fig. 9 (3) and (5) suggests that the three-sided condition of the posterior rectum is due to the reduction of the dorso-lateral and ventral sides to narrow bands; this conclusion is supported by the arrangement of the dilator muscles.

TEXT-FIG. 10.



Tail of caterpillar with the body-wall almost entirely cut away. The posterior dorsal (*P.D.S.*) and ventro-lateral (*P.VL.S.*) muscle sheets are cut across to expose the anterior dorsal (*A.D.S.*) and ventro-lateral (*A.VL.S.*) muscle sheets underneath. *AN.C.*, anal clasper; *AR.*, anterior rectum; *PR.*, posterior rectum. Other references as in Text-fig. 9.

The epithelium is composed of cells rather smaller than those of the anterior rectum (vide table, p. 358). The intima has no bristles.

The musculature is more complicated than that of any other part of the gut and is not easily described. The only transverse muscles present are at the extreme posterior end, thus forming a small anal sphincter (Text-fig. 9 (5)). Longitudinal muscles

passing outside these transverse ones are found on all three faces. On the dorsal side these pass along its whole length, whilst on the ventro-lateral sides they cover only the posterior half (Text-fig. 8).

Owing to the extreme narrowing of the dorso-lateral and ventral faces the six dilator muscles, corresponding morphologically to the six of the posterior sphincter region, are brought together into three pairs. These are to be seen in Text-fig. 9 (4), d_1 , d_2 , and d_3 , corresponding to the similarly lettered muscles of Text-fig. 9 (3). They pass directly outwards from the union of anterior rectum and posterior rectum to the body-wall between body segments nine and ten.

Besides these another system of dilator muscles is to be seen. This system is composed of sheets of muscles passing from the faces of the posterior rectum outwards to the body-wall. At the junction of the anterior rectum and the posterior rectum three wide sheets are to be found passing from the wide ventro-lateral and dorsal faces forwards to the body-wall between segments nine and ten (Text-figs. 9 (4) and 10). These may be named 'anterior dorsal' and 'anterior ventro-lateral' muscle-sheets. Single stout muscles pass from the narrow dorso-lateral and ventral faces forwards to the middle of segment nine. These have their splanchnic attachments between the members of the pairs belonging to the other series (d_1 , &c.) (Text-figs. 9 (4) and 10, *a.d.l.f.m.* and *a.v.f.m.*). These three will be referred to as 'the anterior dorso-lateral face' and 'anterior ventral face' muscles.

At the extreme posterior end of the posterior rectum still more dilator muscles are to be found. Two muscles pass from the dorso-lateral edges directly upwards to the body-wall in segment ten. These are lettered d_1 in Text-fig. 9 (5), because they may correspond to the similarly lettered muscles in Text-fig. 9 (3) and (4). The muscle which seems to correspond to d_2 passes straight downwards; d_3 is absent.

Sheets of muscle fibres pass from the dorsal and ventro-lateral faces, at the extreme posterior end, forwards to the body-wall at the junction of segments nine and ten (Text-figs. 10 and 9 (5)—posterior dorsal and ventro-lateral muscle sheets).

Text-fig. 9 (5) shows a few other muscles (*y* and *z*) attached to the extreme posterior margin of the posterior rectum.

The anus itself is provided with numerous muscles which it seems advisable to consider only in the light of a complete study of the terminal segmentation of the body. As the anus itself has only hypodermal boundaries and is not proctodaeal, none of the muscles have splanchnic attachments, and therefore they do not come within the compass of the present study.

Attachment of Malpighian Tubules.

This matter may be considered here since it concerns structures of proctodaeal origin. In *Vanessa urticae* there are six tubules, three on each side, given off from a pair of common ducts which open into the hind-gut. The common ducts pass under the muscles which surround the anterior, sphincter region, and applying themselves to the ventro-lateral faces of this region, pass backwards to open into the commencement of the colon (Text-fig. 9 (1)). A longitudinal section through the common duct and the tubules proper shows that the common duct is composed of large cells similar in general appearance to those of the hind-gut epithelium. Near where this joins the tubule is a ring of small cells similar in appearance to the interstitial rings of the fore-gut and hind-gut. The chitinous intima is continued from the hind-gut over the cells of the common duct and over the ring of small cells, where it ceases. The epithelium of the tubules is composed of cells bearing a prominent striated hem, thereby showing morphological affinity with the mid-gut cells.

Comparison with the junction of the fore-gut and mid-gut or hind-gut and mid-gut suggests immediately that the cells of the tubules have the same embryonic origin as the mid-gut cells, and that the common duct and its ring of small cells (interstitial ring of the Malpighian tubules) are of proctodaeal origin.

It is commonly asserted that the whole of the tubules are of proctodaeal origin, but I believe that only the common ducts have such a derivation and that the tubules are of endodermal affinities.

In my opinion the posterior interstitial ring of the hind-gut

and the interstitial rings of the Malpighian tubules are genetically allied, arising together in the embryo and separating only at a comparatively late phase of development.

This subject will not be pursued further here, but will be left for consideration under the heading of Malpighian tubules.

(b) Development of the Larval Hind-gut.

The main features of the growth of the larval hind-gut are very similar to those of the fore-gut. The differentiated regions grow by increasing the size of their constituent cells, whilst the cells of the posterior interstitial ring continuously undergo mitosis and remain small. The only really marked difference between the growth of the fore-gut and the hind-gut concerns what happens during ecdysis. Certain parts of the fore-gut seem to expand very considerably at the moult as if they were being held by the chitinous intima. This is not shown in the hind-gut, where the increase in size of the various regions is continuous.

The Posterior Interstitial Ring has many features of similarity with the anterior ring.

The rate of division of its cells seems to be less in the posterior layer than in the anterior one, and with this is associated the fact that the nuclei of the posterior layer are bigger than those of the anterior layer. Its growth takes the form of continued increase in diameter and height of the folds—no new cells are added to the ileum. The table on p. 357 gives data with regard to the growth of the ring in larval phases, and figs. 9a and 9b, Pl. 14, show the form of the ring at different stages of larval life.

The Ileum and Anterior Sphincter region grow merely by increasing the size of their constituent cells. The nuclei of the ileum tend to become rather flattened and apparently larger than those of the sphincter region.

In the case of the Colon the epithelium becomes composed of columnar cells by the end of the first stadium (Text-fig. 8), and only takes on its characteristic form after the first ecdysis. The nuclei are very large in the fifth stadium, 40–60 μ in diameter according to the degree of their flattening.

Exactly the same principles are found in the Anterior

and Posterior Rectal Regions. After the second ecdysis there is noticeable difference in size between the nuclei of the two regions (vide table, p. 358).

A summary of the data obtained with regard to the growth of the larval hind-gut is given on p. 358.

Growth of Posterior Interstitial Ring.

Dimensions in μ .

Phase of Life History.	Length of anterior layer.	Length of posterior layer.	Dia- meter of ring.	Nuclear Diameter.	
				Anterior layer.	Posterior layer.
Early first instar	30	30	60	3-4	3-4
Late first instar	40	40	200	5	5
Ecdysis period	—	—	—	—	—
Early second instar	40	40	230	4-6	5-6
Late second instar	50	50	300	4	5-6
Ecdysis period	—	—	—	—	—
Early third instar	50	50	400	4	5-6
" "	60	60	540	—	—
" "	80	80	650	4	5
Late third instar	50	50	530	4	5
Very late third instar	60	140	360	4	5-6
Ecdysis period	—	—	—	—	—
Early fourth instar	85	110	400	4	5-6
" "	70	140	400	—	—
" "	65	140	470	5-6	8-10
" "	110	160	720	4	7-10
" "	70	130	900	4	5-6
Late fourth instar	110	140	600	4	5
Ecdysis period	—	—	—	—	—
Early fifth instar	100	160	600	5	5-7
" "	90	190	600	5	5-7
" "	100	120	900	5	7-10
Late fifth instar	120	120	1,700	5	5-7

Growth of Hind-gut.

Dimensions in μ .

Phase of Life History.	Diameter of nuclei.						
	Int. Ring.	Ileum.	Antr. Sphinc- ter.	Colon.	Post. Sphinc- ter.	Antr. Rec- tum.	Post. Rec- tum.
Early first instar	3-4	3-4	4	4	4	3-4	3-4
Late first instar	5	5-6	5-6	6-7	6	6-7	5-6
Late second instar	4	10-15	8-12	8-10	10	8-10	8
Late third instar	4	15	10-20	25-30	20	30-40	15-20
Early fourth instar	4	20	10-20	30	20-30	35-40	15-20
Late fourth instar	4	30-35	10-25	30-40	30	30-60	20-35
Early fifth instar	5	30-40	10-25	40-50	30-40	40-60	20-35

GENERAL SUMMARY.

(1) The main features of the anatomy, histology, and development of the larval gut are described.

(2) The fore-gut is divisible into pharynx, oesophagus, crop, oesophageal valve, and anterior imaginal ring, or as here called, anterior interstitial ring. Details of the structure of each part are given, including an account of the musculature.

(3) The growth of the fore-gut is then traced and it is shown that increase in size is accompanied by increase in size of the constituent cells. Only in the interstitial ring does cell division occur after hatching.

(4) A considerable degree of histological differentiation is only to be observed after the commencement of feeding.

(5) The larval development of the mid-gut has been described in my previous paper (vide Bibliography).

(6) Evidence is presented for regarding the peritrophic membrane as entirely derived from the mid-gut.

(7) The hind-gut is then described as consisting of posterior interstitial ring (posterior imaginal ring), ileum, anterior sphincter region, colon, posterior sphincter region, anterior rectum and posterior rectum. The musculature of both fore-gut and hind-gut is shown to be based on a radially symmetrical system of sixes.

(8) The growth principles of the hind-gut are essentially similar to those of the fore-gut.

(9) Evidence is presented to suggest an endodermal derivation for the malpighian tubules.

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EXPLANATION OF PLATE 14.

LETTERING.

AB.F., abaxial fold; *AD.F.*, adaxial fold; *A.D.S.*, anterior-dorsal muscle sheet; *AN.*, anus; *2nd ANT.D.*, second anterior dorsal dilator muscle; *ANT.V.*, anterior ventral dilator muscle; *A.R.*, anterior rectum; *A.V.F.M.*, anterior ventral face muscle; *D.*, dorsal; *d₃*, ventro-lateral dilator muscle; *D.L.*, dorsal longitudinal muscle; *FD.*, food in gut lumen; *F.G.*, frontal ganglion; *IL.*, ileum; *IMAG.*, imaginal cell; *INT.*, intima; *M.*, mandible; *MAL. TUB.*, terminal convolutions of the Malpighian tubules on the anterior rectum; *M.G.*, mid-gut; *MID.V.*, middle ventral dilator muscle; *OES.*, oesophagus; *OES.D.*, oesophageal dorsal dilator muscle; *OES.V.*, oesophageal valve; *P.D.S.*, posterior dorsal muscle sheet; *PH.*, pharynx; *P.I.R.*, posterior interstitial ring; *P.M.*, peritrophic membrane; *P.R.*, posterior rectum; *P.S.R.*, posterior sphincter region; *V.*, ventral; *V.L.*, ventral longitudinal muscle.

All figures drawn with camera lucida.

Fig. 1.—Longitudinal sagittal section of the pharynx of an early fifth instar.

Fig. 2.—Transverse section of the pharynx of a late second instar.

Fig. 3.—Longitudinal sagittal section of the oesophagus of an early fifth instar.

Fig. 4.—Transverse section of the oesophagus of a late second instar.

Fig. 5.—Transverse section of the oesophageal valve of a late third instar.

Fig. 6.—Longitudinal section of the oesophageal valve and anterior interstitial ring of a late first instar.

Fig. 7.—Longitudinal section of the anterior interstitial ring of an early fifth instar.

Fig. 8.—(a) Pharyngeal epithelium of an early first instar.

(b) Same, of a late first instar.

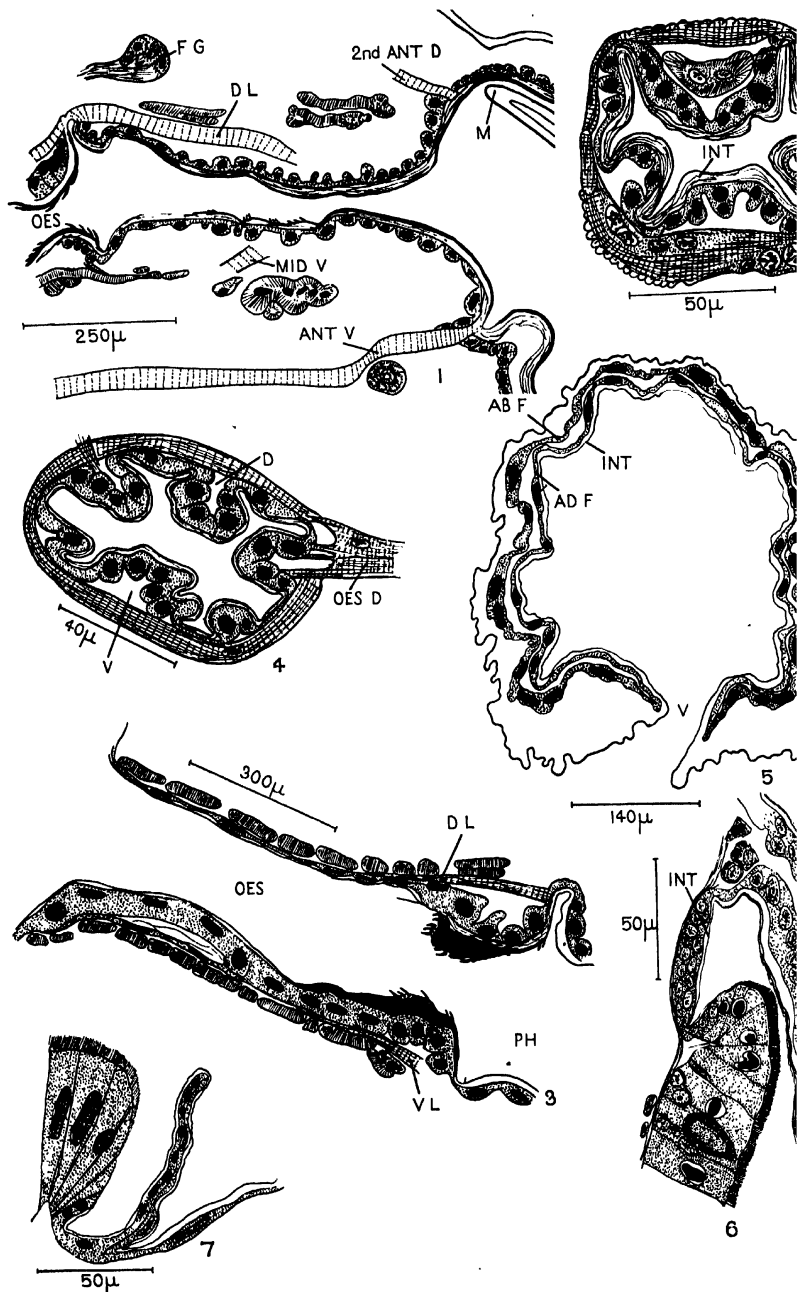
Fig. 9.—(a) Longitudinal section of the posterior interstitial ring of a third instar.

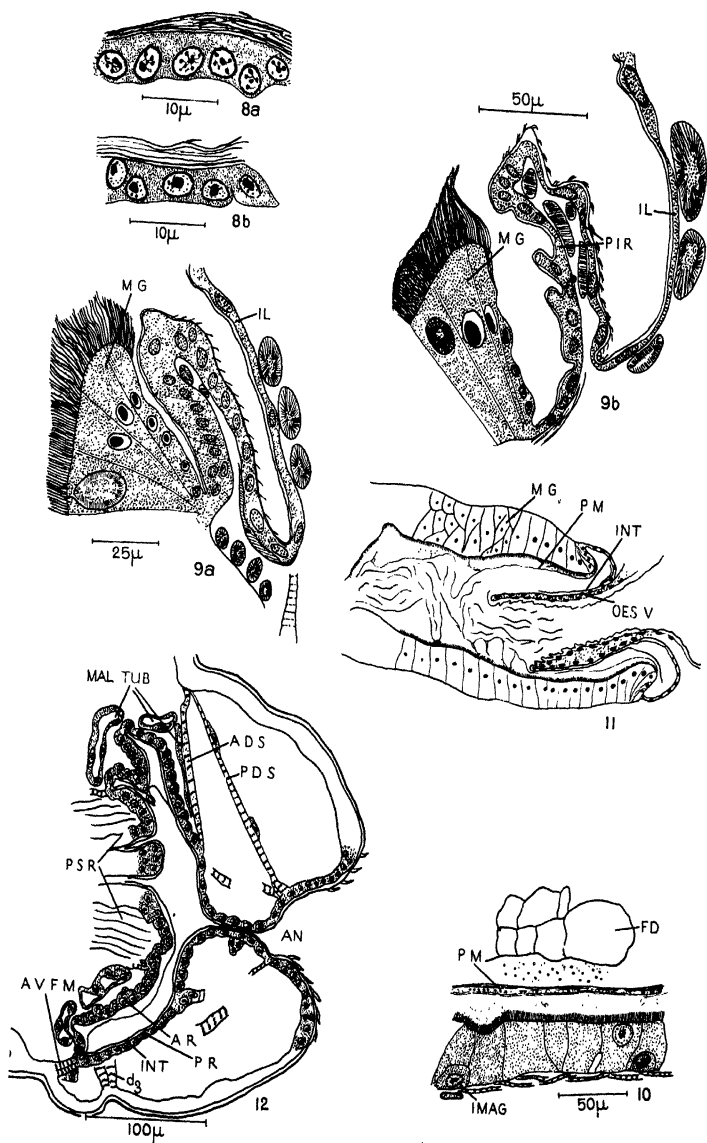
(b) Same, of an early fifth instar.

Fig. 10.—Transverse section of part of the mid-gut and peritrophic membrane—fifth instar.

Fig. 11.—Longitudinal section of the anterior part of the mid-gut to show the peritrophic membrane.

Fig. 12.—Longitudinal sagittal section of the rectum and anus of a late first instar.





The Club-shaped Gland of *Amphioxus*: (a correction).

By

Edwin S. Goodrich.

Professor W. Garstang has pointed out to me that I misinterpreted his meaning when, in my recent paper on "The development of the Club-shaped Gland of *Amphioxus*" (this Journal and volume, p. 155), I stated that he 'appears to be under the erroneous impression' that the bulk of the club-shaped gland develops from the left and not from the right side (p. 157). My interpretation was based on his statements that the gland 'is essentially left-sided', and that 'the bulk of the gland is derived from the left, not the right, side' (p. 140 of his paper on "The morphology of the Tunicata, and its bearings on the Phylogeny of the Chordata", 'Quart. Journ. Micr. Sci.', vol. 72).

I regret that at the time I did not realize that he was referring not to the topographical but to the 'morphological' left side of the embryo, although on re-reading the passage as a whole I now see that this was undoubtedly his intention.

Professor Garstang's position is that since the bulk of the gland develops behind the longer morphological left limb of the endostyle it belongs mainly to the morphological left side of the body, and cannot, therefore, be regarded as a gill-slit of the morphologically right series, as maintained by Willey and van Wijhe.

My own view is that the duct, which arises in the topographical mid-ventral line, may perhaps represent an anterior 'primary' morphologically left gill-slit, but that the evidence is not clear that the glandular part belongs more to the morphological left than to the right side.

It may be added that Professor Garstang now considers that my description of the development of the club-shaped gland lends support to his suggestion that there is a phylogenetic connexion between this gland of *Amphioxus* and the left gill-slit and ventral 'pharyngeal packet' of Appendicularians.

To Professor Garstang I am indebted for pointing out an error in my Text-figure 5, where *rw* should read *lw*.

The Scientific Work of Edwin Ray Lankester

THE death on August 15, 1929, of Sir E. Ray Lankester, formerly Editor of this Journal, was announced in the November number of vol. 73, 1929.¹ Lankester's long connexion, as Co-Editor, as sole Editor, and finally as Honorary Editor, with the 'Quarterly Journal of Microscopical Science' has already been dealt with by A. Sedgwick and W. F. R. Weldon in the complimentary number dedicated to him on the occasion of the twenty-fifth year of editorship (no. 141, vol. 36, 1894), and again on his seventieth birthday by G. C. Bourne (no. 253, vol. 64, 1919).

In the following pages an attempt is made to give an account of his published work. Lankester was a prolific writer. In the course of a long life he wrote some two hundred papers and books. Most of his papers are quite short, many are beautifully illustrated by his own hand. Only the more important of these works can be mentioned here.

When only sixteen years of age Lankester wrote his first contribution to this Journal, 'On our present knowledge of the Gregarinidae, with descriptions of three new species belonging to that Class', vol. 3, 1863. It is a remarkably clear historical account of the work on a group of Protozoa in which he never ceased to take an interest. It is not from lack of encouragement from him that the life-history of the few species he described from a Serpulid, a Sabellid, and Aphrodite have not yet been worked out. Lankester gave a popular account of

¹ General obituary notices appeared in 'The Times' of August 16, 1929, in 'Nature' of August 24, 1929, in the 'Journal of the Marine Biological Association', vol. 16, 1930, in the 'Proceedings of the Royal Society', B, vol. 106, 1930, and in the 'Journal of the Royal Microscopical Society', vol. 49, 1929. It may be mentioned that in the first and in the last of these notices the date of Lankester's resignation of the Linacre Chair in Oxford to go to the British Museum is wrongly given as 1892 instead of 1898.

the parasites of the Cockroach in 1865, and wrote several other papers on Gregarines during the following years, the most important being perhaps his observations on the parasites of *Sipunculus* made in Naples, 1873. In his masterly article on the Protozoa in the ninth edition of the 'Encyclopaedia Britannica', 1885, Lankester divided the Gregarines into two Orders: the Haplocyta including monocystid forms, and the Septata including the genus *Gregarina* and other polycystid forms. No better way of classifying the group has yet been found. So early as 1866 he was searching for a sexual mode of reproduction in Gregarines, and became convinced that the process must occur in the spore stages. This misconception seems to have led him later to propound theories of the nature of the sporozoites of *Haemamoebae* which have proved to be erroneous.

It was Lankester who recognized that the trypanosome of the frog is a protozoon with an undulating membrane and named it *Undulina* ('Q.J.M.S.', vol. 11, 1871). He remarked that it was 'extremely likely' that this parasite had been seen before, and in fact it had been observed some thirty years previously and was named *Trypanosoma* by Gruby in 1843. An intracorpuseular protozoan parasite seems to have been first recorded by Chaussat (*Anguillula minima*, 1850) from the blood of the frog. Lankester rediscovered this minute parasite, 1871, recognized it as a Protozoon and named it *Drepanidium ranarum*. Later he placed it among the Sporozoa, 1882, and here, with other Haemogregarines, it remains under the name *Lankesterella*.

Lankester further described several new or little known Ciliata, Lobosa, and Foraminifera. His beautifully illustrated paper on *Haliphysema* ('Q.J.M.S.', vol. 19, 1879) may be mentioned as a model of lucidity; it fully confirms Saville Kent's view that this form is a foraminiferon and not a sponge as held by Haeckel.

Nor was his work confined to the Protozoa among Protists, but extended to the Schizophyta and lower plants. Besides observations on *Archerina* (*Micractinium*), *Golentinia*, and *Botryococcus*, 1885, 1908, may be mentioned particularly

a paper 'On a Peach-coloured Bacterium' ('Q.J.M.S.', vol. 13, 1873), in which Lankester first formulated the doctrine of pleomorphism sometimes wrongly attributed to Nägeli and others. Having shown that this bacterium, with its characteristic pigment, exists under different form-phases and that intermediate transitional forms occur, he maintained that 'the natural species of these plants are within proper limits "Protean"', and further that various cocci, bacilli, vibrios, spirilla, and leptothrix forms are but form-phases of such protean species found under different conditions. Lankester concluded that true species of Schizophyta must be defined not by simple form-features, 'but by the ensemble of their morphological and physiological properties as exhibited in their complete life-histories'.

Lankester's original work on Coelentera was not very extensive; he, however, contributed an excellent article on the 'Hydrozoa' to the 'Encyclopaedia Britannica', 1881. In 1880 he published papers on the newly discovered freshwater medusa *Limnocoedium* (*Craspedocustes*) *sowerbii*, and later an elaborate study of the processes of intracellular digestion in its endoderm ('Q.J.M.S.', vol. 21, 1881). About the same time he made interesting comparisons between the larval stages of *Geryonia* and the *Ctenophora*, 1881.

Very early in life Lankester developed a keen interest in Annelids. When a mere schoolboy he gave a good account of the 'red-worm', *Tubifex rivulorum* in the 'Popular Science Review' (1863). Shortly after there followed a work on the anatomy of the earthworm *Lumbricus terrestris* ('Q.J.M.S.', vols. 4 and 5, 1864, 1865), a truly remarkable production from the pen of so young an author, combining the scattered observations of previous authors, more especially of D'Udekem, Hering, de Quatrefages, Milne-Edwards, Clarke, and Busk, with his own results into an admirably complete description of this important type. Having confirmed Hering's account of the structure of the genital organs, Lankester discussed the relation of genital ducts to 'segmental organs' (nephridia as he afterwards called them). Claparède had concluded that in *Limicolae* (where nephridia are absent in the

genital segments) the genital ducts must be modified segmental organs. Lankester, impressed by the fact that normal nephridia coexist with genital ducts in the genital segments of Terricolae, suggested that the Oligochaeta were originally provided with two pairs of tubules in each segment, and that while one series survived in the genital segments only of both groups to form the ducts the other series persisted in these segments only in the Terricolae. But this ingenious suggestion has not stood the test of time, and the theory that the genital ducts in Oligochaeta, and indeed in Ceelomata generally, are modified nephridia was abandoned by him when it was shown many years later that they are organs of different origin, 1900. It should be mentioned that Lankester proved, in 1878, that colourless corpuscles are present in the red blood of the earthworm.

In later studies on the lower Oligochaeta, 1869-71, he showed that there are distinct 'larval' and sexual forms, and that when the larval gemmiparous form is transformed into the sexual form new special 'genital' chaetae may be intercalated in certain anterior segments.

His larger memoir on the 'Lower Annelids' read before the Linnean Society in 1867, but not published till 1870, contains an elaborate account of the chaetae, reproductive organs, and other parts of *Aeolosoma* and *Chaetogaster*, and an analysis of their mode of reproduction by fission. Developing Herbert Spencer's theory of the integration of secondary aggregates, Lankester concludes that 'there is a longitudinal cohesion or integration which counteracts this' tendency to produce a head seen in fission. 'When the longitudinal cohesion becomes weak, the tendency to anterior development prevails, and the normal head grows [forwards] and a new individual separates'. It is interesting to compare this tentative explanation of segmentation and budding with modern theories of axial gradients.

The formation of the spermatophores in Tubificids also attracted his attention, 1870, 1871. These remarkable structures, looking and behaving so like Infusoria, he showed to be moulded in the neck of the spermatheca. It was not till much later that Lankester again took up the study of Annelids when

he published new observations on the microscopic anatomy of the Leech, 1880, made with his pupils, J. E. Blomfield and A. G. Bourne, which led to the appearance of the latter's monograph on the Hirudinea in 1884 ('Q.J.M.S.', vol. 24). The fine network of capillary vessels, no doubt respiratory in function, was described running between the epidermal cells; also new light was thrown on the structure and function of the botryoidal tissue, the connective tissue, and the nephridia.

His contributions on the Lithodomous Polychaeta (*Sabella* and *Leucodore*) which bore their way through limestones with the help of acid secretions; on *Thalassema*; on the structure of the large female and small male *Hamingia*; on *Sipunculus*, with a description of the origin of its strange free-swimming ciliated 'urns' developing from the coelomic epithelium, may here be mentioned. More noteworthy, perhaps, are his papers on the genus *Rhabdopleura*. Much interested in this scarce and little-known form Lankester first wrote on its affinities in 1874, and ten years later gave a beautifully illustrated account of its structure, mode of growth, and of budding ('Q.J.M.S.', vol. 24). With regard to its affinities, Lankester eventually adopted the comparison made by Caldwell, and in his article on the Polyzoa, written for the ninth edition of the 'Encyclopaedia', 1885, founded a group *Podaxonia* including not only the Pterobranchia (*Rhabdopleura* and *Cephalodiscus*) but the Sipunculoidea, Brachiopoda, and Polyzoa as well. Although this grouping has by no means been universally accepted, it would seem (with the exception of the inclusion of the Entoprocta) to be well-justified.

A paper 'On some undescribed points in the Anatomy of the Limpet, *Patella vulgata*', published in 1867 (Ann. Mag. N.H., vol. 20), is his first work dealing with the Mollusca. In it are described a small left and a larger right kidney, and the reno-pericardial duct. This discovery of paired kidneys was later confirmed by V. Jhering and extended to *Haliotis* and *Fisurella*, and led Spengel to formulate his theory of the torsion of the visceral hump of Gastropods. It may be considered as the first step in the elucidation of this interesting problem.

Lankester's studies on the early development of *Loligo* and various Gastropods and Lamellibranchs (1871-7) brought out many new and important results. With the very limited technical means at his disposal, he described the ovum, its cleavage, the formation of the germ-layers, the influence of yolk on development, the inpushing of the hypoblast by 'invagination', the closure of the orifice of invagination for which he proposed the name 'blastopore' ('Q.J.M.S.', vol. 15, 1875), the formation of a closed two-layered 'planula', the fact that in *Paludina* the blastopore coincides in position with the anus, the ingrowth of the ectoderm at the mouth to form an ectodermal region of the alimentary canal he later called the 'stomodaeum' (1878) and a similar ingrowth at the anus to form the 'proctodaeum', the multiple origin of the mesoblast, and the development of a trochosphere stage, with preoral ciliated ring later to become the 'velum'. His discovery of the early-formed 'shell-gland' enabled him to define the important later 'Veliger' larval stage characteristic of Mollusca in general.

From these embryological researches and those of Kowalevsky and other contemporary workers, Lankester drew material for two noteworthy essays, landmarks in the history of Embryology. The first, founded on lectures delivered in Oxford and published in 1873 ('Ann. Mag. N.H.', vol. 11), but the substance of which was written before the appearance of Haeckel's famous 'Monograph on Sponges', 1872, and *Gastraea* theory, 1873, is entitled 'On the Primitive Cell-layers of the Embryo as a Basis of Genealogical Classification of Animals, and on the Origin of Vascular and Lymph Systems'. Having reviewed the relations of Evolution to phylogeny and ontogeny, and described the development of Metazoa from the single cell through the 'Polyplast' stage (Morula of Haeckel) to the 'Planula' (Gastrula of Haeckel) either by direct growth (later called 'delamination') or by invagination, he divides multicellular animals into 'Diploblastica' (Coelentera and Sponges) and 'Triploblastica'. This latter name for the forms provided with a mesoblastic layer is really preferable to Coelomata, later adopted, since the coelomic body-cavity is scarcely recognizable in the lower

groups. Lankester then maintained that the Planula is common to all the Metazoa, that there is no fundamental difference between its origin by invagination and by direct growth or delamination, and that the former method may well be due to a shortening of the developmental process. (Invagination is an economy of material, a mode of rapidly filling in the outline of an organ in the embryo leaving it in a hollow condition for subsequent completion, 1874.) He pointed out that whereas the otocyst and to some extent the ganglia arise by invagination in *Loligo*, in Nudibranchs and other Gastropods they may arise as solid thickenings. The relation of the various tissues to the germ-layers, the development of blood and lymph spaces in the mesoderm, the origin of corpuscles from their walls, the distribution of segmental organs, are discussed. A figure of the 'Archiscolex' or primitive worm-like form is given, no mean precursor of the more famous 'Archimollusc'. A very important passage occurs in the discussion on the nature of the Prostomium: 'The segmentation of the prostomial axis in Arthropoda and some Annelids, which has an appearance of being a zooid-segmentation comparable to that of the metastomial axis on account of the identity in the character of the appendages with those of the metastomial axis, has yet to be explained. It may be suggested that it is due to a distinct breaking up of this axis like the posterior one into zooid-segments: there is much against this supposition. Much more likely, it seems, is the explanation that the oral aperture shifts position, and that the ophthalmic segment alone in Arthropoda represents the prostomium, the antennary, and antennular segments being aboriginally metastomial and only prostomial by later adaptational shifting of the oral aperture'. This bold suggestion, as we shall see later, was to give the clue to the morphology of the Arthropod head.

The second essay, 'Notes on the Embryology and Classification of the Animal Kingdom, comprising a Revision of Speculations relative to the Origin and Significance of the Germ-layers', contains an elaboration of some points and a revision of some conclusions ('Q.J.M.S.', vol. 17, 1877). By this time Haeckelism dominated embryology; but, although Lankester greatly

admired some of Haeckel's zoological work, he preserved his independent judgement. He did not accept the *Gastraea* theory, pointed out objections to it, maintained that the blastopore is not identical with and is not represented by the mouth as held by Haeckel and Huxley, that the gastrula with its archenteron can be formed by delamination (as in *Geryonia*) or by invagination, and that the former is probably the more primitive method. Figures are given in this essay to show the possible phylogenetic stages from a 'monoplast' ingesting solid particles of food, to a polyplast or morula in which the particles pass to the interior, and finally to a diblastula in which the food enters a central digestive cavity surrounded by endoderm. Stomodaem and proctodaem erupting into the enteron are later formed in relation to mouth and anus. Regarding the blastopore as an orifice of secondary nature existing temporarily and solely in relation to the invagination process, he explains its occasional coincidence with mouth or anus as cases of secondary adaptation. A new and important conception of 'precocious segregation' is invoked to explain differentiation of the blastomeres in cleavages, and differences between epibolic and embolic invagination.

Discussing the origin of the coelom this principle of Precocious Segregation is again called upon to explain its different modes of development. Huxley had distinguished between enterocoel and schizocoel. Lankester here maintains that there is no fundamental difference between the nipping off of hollow diverticula of the archenteron and the hollowing out of the solid mesoderm. Less happily he further holds that the former is the more primitive mode of origin; a view inconsistent with the modern Gonocoel theory which seems to be far nearer the truth.

It is in this essay that Lankester introduced his well-known nomenclature for the various parts of the vertebrate kidney; the whole individual series of tubules he called the archinephron with its longitudinal archinephric duct, while to the successive portions which succeed each other in development he gave the names pronephron, mesonephron, and metanephron with their respective ducts. Here also he proposed the name nephridium for the excre-

tory tubules generally known as segmental organs and for the genital ducts throughout the Coelomata. It was then held that these excretory and genital ducts are all more or less modified nephridia; but, when it was shown many years later that two quite different sets of organs, the true nephridia and the coelomoducts, had been confused under the one name, Lankester readily accepted the new interpretation, 1900. ('Treatise on Zoology', Part 2.)

After discussions of various morphological subjects including larval forms and the modifications of the primitive ciliated band or 'architroch', the paper ends with a tabular statement of classification in which the new term 'Grade' is introduced to indicate marked advance in differentiation along a phyletic branch. These two essays together occupy only some 70 octavo pages.

Among Lankester's other contributions to our knowledge of the Mollusca may be mentioned his exposure of the fallacy that water is taken in and expelled from the vascular spaces, 1884; his observations on the development of the yolk-sac, otocyst, eye, ganglia, and pen-sac of *Loligo*, 1873, 1875; the paper written in collaboration with A. G. Bourne on the 'Osphradium' and genital ducts of *Nautilus*, 1883; and the publication under his direct guidance by his pupil R. H. Peck, 1878, of an important memoir on the gills of *Anodon*. Having described in detail the minute structure of these complex lamelliform gill-plates, it was shown how step by step they could have been derived from the more primitive 'ctenidium' of *Mytilus* by the concrescence of the free axis with the body and of the simple separate filaments with each other.

To the ninth edition of the 'Encyclopaedia Britannica', 1883, he contributed an admirable article on the Mollusca. Although now out of date in some respects, notably in regard to his own later work on the coelom and vascular system, it remains the most comprehensive and illuminating account of the whole group ever written in so short a space. Here is built up the schematic 'Archi-mollusc', not a phantastic ancestor derived from some embryonic or larval stage according to Haeckelian doctrines of recapitulation, but a viable adult creature embodying all the essential organs which, according

to the evidence of comparative anatomy, the primitive Mollusc must have possessed. The modifications of this type in adaptation to various modes of life Lankester traced along the diverging branches of the phylum with convincing lucidity.

His most important work on Crustacean morphology is contained in a paper entitled 'Observations and Reflections on the Appendages and on the Nervous System of *Apus cancri-formis*' ('Q.J.M.S.', vol. 21, 1881). A careful description of the appendages and detailed comparison with those of other Crustacea is followed by an interpretation of the significance of the structure of the nervous system of peculiar interest and novelty. In the description of the nervous system of *Apus* given by Zaddach in 1841 (for Lankester's own specimens were badly preserved), he finds striking confirmation of the suggestion made in his essay of 1873 that there has been a relative shifting backwards of the mouth and forwards of the segmental appendages in Arthropods. For whereas in higher Crustacea both first and second antennae are innervated from the preoral brain, in *Apus* not only the second but even the first pair of antennae is supplied from post-oral ganglia, a condition approached by *Limulus* whose cheliceral nerves come off from the lateral nerve-cords posteriorly to the brain. Lankester concluded that there is a progressive tendency for the cephalization of the anterior segments of segmented animals, that in Arthropods the paired originally post-oral ganglia tend to move forward with their appendages and fuse with the primary median brain, his 'archi-cerebrum', situated in the prostomium; and further, that while this primitive archi-cerebrum remains pure in Annelids, in Arthropods it becomes more and more complex by the successive addition of ganglia from behind to form a 'syn-cerebrum'. This simple but brilliant suggestion, explaining at once the presence of preoral appendages and the complex structure of the brain in Arthropoda, has been generally accepted, and much work has since been done in the attempt to make out exactly how many segments make up the head in the various diverging branches of the phylum.

One of his most remarkable achievements was the proof that *Limulus* is an Arachnid closely related to *Scorpio* in a

celebrated paper entitled 'Limulus an Arachnid' ('Q.J.M.S.', vol. 21, 1881). Straus Durkheim, 1929, had long ago maintained that the 'King Crab' should be placed in the Arachnida, since it has no antennae, legs radiating from a common sternum, and an internal cartilaginous sternum. But, in spite of his arguments, Limulus was always classified either with the Crustacea or in a special group. Lankester, in the paper mentioned above, compared Limulus and Scorpio organ by organ, segment by segment, and with irrefutable logic brought forward overwhelming evidence that these two Arthropods so different in appearance are not merely allied but closely related forms, the one more primitive and adapted to aquatic life, the other more specialized and modified for life on land. The evidence was completed in other publications on the respiratory organs, 1881, the excretory organs, 1882, 1884, the endoskeletal and muscular systems, 1885 (written with the assistance of W. B. Benham and E. J. Beck), and particularly the eyes (with A. G. Bourné), 1883.

In these and a later valuable article on the 'Structure and Classification of the Arachnida', written for the 'Encyclopaedia Britannica', 1902, and reprinted in vol. 48 of this Journal, Lankester showed that the Arachnida form a degenerating series leading from the more primitive fully segmented and earliest forms to the Acari in which segmentation is almost completely lost. These writings not only greatly advanced the knowledge of Arachnids, but incidentally threw welcome light on the evolution of the Arthropoda in general, and the interrelationships of the various groups included in that large phylum, a subject which he discussed in two other publications, 1897, 1902.

We may turn now to his later work on the coelom and vascular system. In a note appended to Gulland's paper on the coxal gland of Limulus and other Arachnida ('Q.J.M.S.', vol. 25, 1885) Lankester compared this gland to the nephridia of Chaetopods and the so-called 'nephridium' of Peripatus, suggested that the genital ducts of Molluscs, Arthropods, and other Coelomata might be modified nephridia, and further maintained that 'the blood-system in larger Arthropoda . . . is altogether distinct from the general system of lacunae of the

connective tissue' and end-sacs into which the excretory tubes open. This important conception led to the enunciation at the British Association meeting at Manchester in 1887 (published in 'Nature', 1888, and the 'Q.J.M.S.', vol. 34, 1898) of one of his most illuminating contributions to Invertebrate Morphology. The nature of the body-cavities in Molluscs and Arthropods had hitherto seemed very obscure. It was generally held that their body-spaces were continuous; vague notions prevailed that the coelom and vascular space had either not yet been differentiated from an ancestral original space, or had become secondarily fused. Lankester showed that in Arthropods and Molluscs the main body-cavity is not coelomic but distended blood-vascular space to which he gave the name 'haemocoel'. By expansion chiefly of the venous vessels the haemocoel has (except in Cephalopods) almost obliterated the coelom leaving remnants here and there. In Molluscs the coelom is represented by the pericardial and perigonadial spaces ('generative glands' or 'gonads'). The kidneys were later recognized as excretory chambers of the coelom. His observations and experiments on the red-blooded *Solen legumen* by injections and silver impregnations showed that complete arteries, veins, and capillaries still persist in certain regions and that coelomic and haemocoelic spaces do not communicate.

In Arthropods, also, the blood-vessels, especially the veins, have swollen to large spaces obliterating the original coelom which persists only in the perigonadial space, and the end-sacs and the small lymph-spaces in the connective tissues into which the excretory tubules open. These conclusions were soon confirmed by the work of Sedgwick on the development of *Peripatus*, of Weldon, Marchal, and Allen on the body-cavity and excretory organs of Crustacea, and of numerous workers on the development of Arthropods.

It was to a great extent Lankester's attempt to understand the origin of the unique structure of the Arthropod heart that led him to this view. This heart, lying in a pericardial blood-space into which it opens by paired ostia, he successfully explained as derived from the Annelid longitudinal dorsal vessel receiving segmental afferent vessels by enlargement and

fusion of the latter vessels, the accompanying obliteration of the pericardial region of the coelom, and the final enclosure of the longitudinal muscular tube in a continuous haemocoelic space. The ostia represent the original afferent openings. Rarely can so short a paper have so successfully solved a number of puzzling morphological problems!

Lankester delighted in the use of the microscope; it was his custom always to examine the body-fluids and blood of the animals he dissected. Hence he made many important observations on the cells and special corpuscles floating in these fluids (1870), but especially did he take up with enthusiasm the new method of examination with the micro-spectroscope introduced by Hoppe Seyler and Stokes, and apply them to the study of pigments, particularly respiratory pigments, in Invertebrates. In these pioneer researches, from 1867 onwards, he established the presence of true haemoglobin in the blood-plasma of the Nemertean *Polia sanguirubra*, of *Lumbricus*, *Eunice*, *Nereis*, and other Annelids, of *Planorbis* among Molluscs, of *Cheirocephalus*, *Daphnia*, and the larva of *Cheironomus* among Arthropods; also in the coelomic corpuscles of *Glycera*, *Capitella*, *Thalassema*, and *Phoronis*, and the Lamellibranch *Solen legumen*. Moreover, he found haemoglobin in the pharyngeal muscle of *Littorina* and other Gastropods, and in the nerve-cord only of *Aphrodite*; and noted that it is absent in the abundant blood-corpuscles of the transparent *Leptocephalus* larva of the eel. In the green-blooded *Chlorhaemidae* he discovered a new respiratory pigment, chlorocruorin, allied to haemoglobin and of similar properties, and a pink pigment in the coelomic corpuscles of *Sipunculus*. In a valuable discussion of the significance of these observations and on the apparently capricious distribution of respiratory pigments ('Proc. R. S.', vol. 21, 1873), Lankester 'suggests the hypothesis of the existence of various bodies not necessarily red, possibly colourless, which act the same physiological part in relation to oxygen as does haemoglobin'—a suggestion which seems to bring him near to Keilin's recent discovery of the existence of a generally distributed cytochrome.

Lankester's interest in these questions lasted throughout his life, and his latest contribution on the subject is a lecture delivered at the Royal Artillery Institution, Woolwich, October 26, 1916, on blood, 'A wonderful fluid' ('Journal of the Royal Artillery', vol. 49).

Here we may allude to Lankester's observations on chlorophyll bodies in *Spongilla* and *Hydra*, which for long he maintained are products of the animals themselves, on 'Green Oysters', 1886, and to his description of 'chaetopterin' from the intestinal wall of *Chaetopterus*, 1897, and 'stentorin' the colouring matter of *Stentor caeruleus*, 1873.

Although the bulk of Lankester's researches were concerned with Invertebrates he did not neglect the Vertebrates. He was much interested in *Amphioxus*, wrote a short paper on it in 1875, and a more serious contribution some years later ('Q.J.M.S.', vol. 29, 1889), in which many new points were described and several excellent diagrams given illustrating its anatomy. However, his 'brown tubes', so-called 'atrio-coelomic funnels', which acquired considerable notoriety, have turned out disappointingly to be merely blind pockets of the atrial wall. In collaboration with his pupil, A. Willey, was published an important description of the later larval development, including a new version of the development of the atrium ('Q.J.M.S.', 1891). This paper together with Willey's previous one on the earlier larval stages stand as classical contributions to our knowledge of *Amphioxus*.

The structure of the heart of *Ceratodus*, *Protopterus*, and *Chimaera*, and of the valves in the conus arteriosus of these fishes was the subject of a communication to the Zoological Society published in 1879.

In a series of three papers on the structure of the heart of Monotremes and *Apteryx*, 1888-5, Lankester corrected a strange mistake made by Owen, and gave an accurate description of the auriculo-ventricular valve of the right ventricle of *Ornithorhynchus* and *Echidna*, pointing out certain primitive characters, and the fact that the almost total absence of a septal flap distinguishes the heart of Monotremes from that of the Ditrematous mammals.

Among other things may be mentioned that the peculiar villi borne on the pelvic fin of the female *Lepidosiren* were first described by Lankester in 'Nature', 1894, and later he published correct descriptions and figures of the externals of *Lepidosiren* and *Protopterus*. The affinities of the interesting carnivore *Æluropus* were the subject of a paper in 1901. His monograph on the newly discovered *Okapia* was never completed; but a part containing many beautiful plates appeared, 1910, and observations on the 'ossicones' or bony cores of the frontal processes, 1901. The comparison of these with the similar processes of the Giraffe and related forms is a valuable chapter in the history of horns and antlers.

Owing possibly to Huxley's encouragement Lankester from his boyhood collected fossils with enthusiasm. The Red Crag of Suffolk was a happy hunting ground where he discovered various new mammalian species and forms new to the locality, 1864-70. But the remains of those earliest Vertebrates now generally known as Ostracodermi attracted him most. A short communication to the 'Geologist' on *Pteraspis*, 1862, is Lankester's first appearance in print. It includes a restoration of the dorsal shields and a figure of some of the pits of the lateral line organs. His later description of scales on the body, 1864, set at rest all doubt as to the piscine nature of these obscure fossils, which had by some been considered as shells of Cephalopods and by others as remains of Crustacea. The publication of his classical monograph on the 'Cephalaspidae' in 1868, marked a great advance in our knowledge of these palaeozoic fishes. Besides giving a systematic and fully illustrated account of all known forms, he described many new species, and gave excellent reconstructions of *Pteraspis* and *Cephalaspis*. Lankester definitely separated the Pteraspids from the Cephalaspids, placing them in two groups which he named 'Heterostraci' and 'Osteostraci' respectively. He considered them to be true fishes, but cautiously concluded that the evidence did not warrant the close association of the Osteostraci, and still less of the Heterostraci, with any known group of Pisces refusing to accept Huxley's opinion that they are allied to the armoured Teleosts. It should be noticed that Lankester had

failed to recognize that the separate shields to which he gave the name *Scaphaspis* are merely the ventral shields of Pteraspids. This was not established till some years later when Kunth and Schmidt found them in association. A remarkable Heterostracan dorsal shield was described, figured, and named *Holaspis* by Lankester in 1873; it shows the complete course of the lateral-line system. Quite lately Stensiö has made use of this figure to support his contention that the Pteraspids are closely allied to the Cyclostomes, a view Lankester had energetically opposed in 1897.

We may now turn to some of Lankester's more general contributions to zoological literature. He had no liking for mere controversy, but was ready to attack what he considered to be false doctrine, and to help in clearing up ambiguities. Speculation remote from facts did not appeal to him, nor far-fetched theories, however popular. He lived through the stirring times when the battle for Evolution was being fought, and was intimately acquainted with many of its most prominent protagonists. A convinced evolutionist and Darwinian, his life-work was to a great extent devoted to the application of the new doctrines to comparative anatomy and embryology, to the tracing of phylogenetic affinities, and the improvement of classification based on evolutionary principles.

In addition to the two essays already noticed, may here be mentioned an important essay on 'Comparative Longevity', 1870, and a lecture on 'Degeneration: a chapter in Darwinism', delivered at the British Association meeting in Sheffield in 1879 (republished in 'The Advancement of Science', 1890). Also his theory of the evolution of blind cave animals, owing to the constant wandering out of the darkness of those individuals which can see best. In an essay, 'On the Use of the Term "Homology" in Modern Zoology, and the Distinction between "Homogenetic" and "Homoplastic" Agreements' ('Ann. Mag. N.H.', vol. 6, 1870), Lankester redefined Owen's terms Homology and Analogy in the light of Evolution. Structures genetically related and traceable to a common ancestor are 'homologous', or, as he preferred to call them, 'homogenous'. Further, parts generally homogenous may, along different phyletic

branches, be differentiated similarly, giving rise to similar parts which are merely 'homoplastic'. Thus, agreement between bones of the skull, or muscles of various regions in different groups may be due to 'homoplasia'. Likewise, the correspondences arising between 'serially homologous' parts are homoplastic. 'Analogy' has a wider meaning, and is applied to any two organs having the same function but no genetic affinity.

In letters to 'Nature', 1894, Lankester discussed 'Lamarckism' and 'acquired characters', and, with his usual insight, went at once to the root of the matter. He pointed out that changes induced in an organism in a new environment are of the nature of responses to the new conditions; that the potentiality to respond of an individual is transmissible by heredity; that 'potential' may be distinguished from 'actual' characters. The term 'acquired character', he held, should be limited to Lamarck's definition indicating acquisitions under new conditions, though 'actual' responsive characters are of the same order as 'potential' characters. He then pointed out that Lamarck's First Law, that new external conditions give rise to new characters, is inconsistent with his Second Law, that such new characters are transmitted by inheritance. 'Since the old character had not become fixed and congenital after many thousands of successive generations of individuals had developed it in response to environment, but gave place to a new character when new conditions operated on an individual (First Law), why should we suppose that the new character is likely to become fixed after a much shorter time of responsive existence or to escape the operation of the first law?' This question still remains unanswered by the supporters of Lamarck.

During his brief residence in Cambridge, before migrating to Oxford, Lankester wrote a paper 'On the Cerebrum of the Entellus Monkey: *Semnopithecus entellus*', 1865. Many years later he returned to this subject in a communication on 'The Significance of the Increased Size of the Cerebrum in Recent as compared with Extinct Animals' read before the Société de Biologie in Paris, 1899. Having pointed out that generally speaking recent Reptilia and especially Mammalia

have larger cerebral hemispheres than their early Tertiary or Mesozoic predecessors, and that a similar but greater relative increase appears when man's brain is compared with that of anthropoid apes, he maintains that, nevertheless, the earlier and more primitive forms cannot be considered as defective in the essential control by the brain, and were no doubt efficient and adequate mechanisms. Man, however, is born with relatively fewer ready-made 'instincts'—performances of inherited nervous mechanism—than lower mammals, but man is endowed with greater capacity for 'learning' and the storing of individual experience, for developing in the course of his individual growth similar nervous mechanisms in response to new and diverse conditions. This 'educability', or adaptability by means of mental powers, is what man possesses in excess as compared with apes. There is a tendency to substitute the more valuable educability for mere inherited brain-mechanisms. Hence selection was probably transferred to the cerebrum, the all-important organ of educability. Although the results of education are not transmissible, yet 'educability' is inherited.

Lankester again developed this theme in his well-known 'Romanes Lecture' delivered in Oxford in 1905.

Before closing this notice something may be said about Lankester's work on Flint Implements. In his later years he became keenly interested in Prehistory and collected objects of the Stone Ages. As an archaeologist he will particularly be remembered for his championship of what he called the 'rostracinate' implement of Pliocene date, first described by J. Reid Moir. Lankester published papers upon this type of implement in the 'Phil. Trans.', B, vol. 290 (1912) and B, vol. 367 (1920), and in the 'Proc. Royal Soc.', B, vol. 91 (1920) and B, vol. 92 (1921), and elsewhere. With characteristic vehemence and detailed argument he supported Reid Moir's contention that the rostracينات were of human manufacture. It may be said that it is in great part due to Lankester's advocacy that these rough and early implements are now accepted as artifacts by a large number of archaeologists.

Little has been said in the foregoing pages about Lankester's more general works. Some of his articles in the 'Encyclopaedia

Britannica' have already been alluded to, but there remain to be mentioned the article on 'Vertebrata', and on 'The history and scope of Zoology', and the very interesting 'Introductions' to Parts I and II of the 'Treatise on Zoology' of which he was general editor.

In his later years Lankester wrote a series of semi-popular books, such as 'Extinct Animals', 'Science from an Easy Chair', 'Great things and Small', full of interesting information.

This brief review of Lankester's writings may help to show how wide was the range of his researches, and how great was the part he played in the progress of zoological science.

EDWIN S. GOODRICH.

The Biological Relationship between *Septobasidium retiforme* (B. & C.) Pat. and *Aspidiotus Osborni* New. and Ckll.

By

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With Plates 15-19 and 60 Text-figures.

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¹ The scale insects have been identified by Dr. Harold Morrison, Chief of Taxonomic Investigations, Bureau of Entomology, U.S.D.A., Washington, D.C. Dr. Morrison has also helped me with the literature on scale insects. In the preparation of the plates and figures I have received help from several sources. Miss Alma Holland inked most of the Text-figures, and part of Plate 19. Mrs. E. R. Couch inked a number of the Text-figures and Plate 16. Dr. J. M. Valentine retouched Plates 17 and 18. It is also a pleasure to thank the editor, Professor E. S. Goodrich, for his kind advice in the arrangement of the figures and plates and for retouching several of them.

Paper presented at the International Botanical Congress, Cambridge, England, August 19, 1930.

I. INTRODUCTION.

ONE of the most unusual and interesting genera of fungi is *Septobasidium*. Allied to the mushrooms through the Thelephoraceae on the one hand, by the flattened resupinate habit and exposed fruiting surface, and to the rusts on the other hand by the similarity of their reproductive cells, the genus occupies a very anomalous position taxonomically.

Though interesting from this standpoint, the genus is of far greater interest in its biology. The seventy odd species, which have so far been described, grow, as a rule, in flattened patches over the bark and leaves, and sometimes the roots of living plants. In some species the fungus growth is so extensive as to clothe the leeward side of the trunk and the under side of many of the branches of trees fifteen metres high (Petch, 1911, 1921, and Couch, 1929). The majority of species, however, grow in smaller patches. In spite of such extensive growth, observers have so far reported, in the great majority of species studied, that the fungus does not penetrate into the living tissues of the plant. If the upper regions of the fungus are scraped away with a knife, numerous scale insects are exposed. Some of the scale insects are imbedded in the tissue of the fungus; others adhere to the wood. Some of the insects are dead, their remains appearing only as empty shells; others are alive. Usually the insects, whether dead or alive, are very abundant. No one can observe such an association of fungus and insects on a living tree without propounding the question as to how the fungus is nourished. Several explanations have been offered.

II. HISTORICAL.

By some observers the fungus has been regarded as a parasite, causing peculiar wounds (Galloway, 1890) in the wood in some species, and witches' brooms in other species (Rick, 1906, 1907; see also Sawada, 1911; Jaap, 1916; Gäumann, 1927). Others have considered the genus as epiphytic (Patouillard, 1892).¹

In 1907, von Höhnelt announced his discovery of scale insects beneath the stroma of *Septobasidium abnorme* (P. Henn.) v. H. et L. Von Höhnelt found, furthermore, that the

fungus did not penetrate at all into the tissue of the leaf. He states, therefore, that if the same condition can be found in other species, there can be no doubt that a real biological relationship exists between the species of *Septobasidium* and the scale insects. 'It may be parasitism or saprophytism, or it may be that a complicated symbiotic relationship exists' [between the scale insects and *Septobasidium*]. Since this very remarkable discovery, by far the greater majority of species which have been described have been found associated with colonies of scale insects; and the species described before 1907, upon reinvestigation, have also been found to be associated with scale insects. Petch (1921), who has studied entomogenous fungi in Ceylon for a number of years, states that all species of *Septobasidium* so far found in Ceylon are associated with scale insects. Indeed, there is only one species of *Septobasidium* which is known not to be associated with scale insects. This species was collected in Jamaica by W. R. Maxon (Couch, 1929), and grows on the fertile side of fern sporophylls, parasitizing the young sporangia.

Since von Höhnel's discovery in 1907, attention has been directed to the relationship between the fungus and scale insects, rather than to the relationship between the fungus and the tree (or other host plant). One group of investigators claim that the fungus parasitizes the bodies of the scale insects, completely overgrowing and finally destroying them (Petch, 1911; Burt, 1916; Coker, 1920; Snell, 1922; Boedijn and Steinmann, 1930); the other claims that, as far as is known at the present time, *Septobasidium* lives saprophytically or epiphytically on the excretions of scale insects (Gäumann, 1926). I shall review only the evidence for parasitism, since Gäumann does not present any evidence in support of the view stated in his text-book.¹

Petch (1911), in a brief note on the biology of the genus

¹ It should be borne in mind, however, that Gäumann (1922), in his extended research on *S. borgoriense* Pat., was not attempting to discover the fungus-insect relationship, but was rather trying to determine if the fungus would grow on a large number of trees and shrubs without insects (see p. 428 below).

Septobasidium, states: 'From an examination of a long series of specimens, it has been determined that these Fungi are parasitic on colonies of scale insects, which they overgrow and completely destroy. . . . These Fungi live, not on the secretions of the insects, as in the case of *Meliola*, but upon the insects themselves.' Again, in 1921, p. 84, Petch makes essentially the same statement.¹

Burt's (1916) observations point to an association of *Septobasidium* with scale insects, and he seems inclined to follow Petch in believing that the fungus completely overgrows and destroys the insects. Burt states that Mr. Seagle from North Carolina wrote that the old fructifications of *S. pseudopedicellatum* disappeared from his apple trees in late spring and early summer, and new fructifications grew, which became large by early winter. I have found, contrary to Mr. Seagle's observations, that *S. pseudopedicellatum* Burt is perennial on several varieties of oak, ash, black gum, pear, and other trees. Indeed, I have had several individual colonies of *S. pseudopedicellatum* Burt marked for three years.

Coker (1920) reporting on *S. pseudopedicellatum* Burt and *S. retiforme* (B. and C.) Pat. states, 'This species attacks colonies of scale insects on living bark;' and, farther on, he says, 'the plant is parasitic on the insects only and does not injure the tree.'

Snell (1922), describing *S. pinicola* Snell on *Pinus strobus*, says that the fungus lives as a pure epiphyte on the tree, but is associated with scale insects, parasitizing and destroying them, as cursory observations reveal. About the nature of this association, Snell says, 'The insects are overgrown

¹ Petch (1921) states, however, that there are certain exceptions to the rule, that some species after destroying the scale-insects attack the plant. Gäumann (1927) in a brief note 'On the parasitism of *S. bogoriense* Pat.' states that he found this fungus growing on hybrid roses imported from Europe in such luxuriance as almost to exterminate the roses. He makes no statement about the fungus-insect relationship but states that the fungus is parasitic on the rose. This is particularly interesting in view of the fact that in an earlier extended research of Gäumann's (1922) he reports that *S. bogoriense* grew very poorly on trees and shrubs without scale insects.

and intergrown with mycelium of the fungus, which can be determined to be within their bodies. The hyphae in the youngest insects were hyaline and on the older ones were dark like that of the context.' Snell presents a photograph (fig. 2, Pl. 12) to show this condition. An examination of material sent me from Pennsylvania by Professor Overholts has shown, however, an entirely different condition from that described by Snell. In spite of the fact that this material was examined two weeks after it was collected, and hence was almost completely dried out, it showed beneath the fungus stroma: (1) non-parasitized insects; (2) non-parasitized adults containing young; (3) parasitized insects. The latter contained numerous haustoria of the fungus in the shape of much gnarled coils, which resemble, in a striking manner, a glomerulus of the kidney. These haustoria within the parasitized insects' bodies are unmistakable, and it is evident from Snell's statements and photograph that he did not recognize the true haustoria of *S. pinicola*.

In 1930, Boedijn and Steinmann, working in Java, report as follows about the fungus-insect relationship: 'As a result of our studies of several species, and of a great amount of material, we have found that these fungi are parasitic on scale insects, living not on their secretions, as is the case with sooty moulds, but on the insects themselves, which they overgrow and finally destroy. The scale insects turn out in a granulous mass, and if the fungus is scraped off, the remains of the insects will always be found on the stem beneath the fungus covering.' And farther on, 'It is very easy to follow the penetration of the fungus into the body of the Coccidae. Hyaline, and sometimes strongly curved hyphae, penetrate the body of the scale insects, filling it up completely, whilst the hyphae assume a more brownish colour. On all species under observation, the Coccidae proved to be enclosed in a very compact layer of mycelium. In the case of *S. tuberculatum*, moreover, the peculiar bubble-shaped cells described above, were frequently found in the bodies of the scale insects.'

From the several quotations just given, it is apparent that none of the observers has made a very detailed study of the relationship between the scale insects and the fungus. It is

possible, I believe, to reconcile, by a simple explanation, the conflicting views of Petch, and the other adherents to the theory of parasitism, with the view of Gäumann, who believes that the fungus lives as a saprophyte on the excretions of the scale insects. All specimens of *Septobasidium* which I have studied, both from temperate and tropical zones, are perennial; that is, individual specimens of the fungus live not for a few weeks or months or even for one season, but rather for years. Indeed, the life of the fungus is normally terminated only by the death of the tree. Moreover, the association between the fungus and scale insects is perennial. If one examines a specimen of *Septobasidium* just after the females have given birth to young, he finds the empty remains of the exhausted bodies of the old females more or less overgrown by the fungal threads. The young, living, healthy insects, at this stage, are so inconspicuous as to be easily overlooked. From such a spectacle as the exhausted bodies of the females overgrown by the fungus, one might naturally conclude that the fungus 'overgrows and completely destroys whole colonies of scale insects'. Such a conclusion as the above might easily be made from an examination of even fresh material. Now, if one makes a cursory examination of a fresh specimen during certain seasons of the year, before the young have been born, and when the adults are abundant, one finds the comparatively large, plump bodies of the adults more or less covered by the fungus but apparently untouched and unharmed by it. It is natural to conclude from such an observation that the fungus lives saprophytically or epiphytically on the excretions of the scale insects.

III. THE PROBLEM.

It is obvious, from the foregoing résumé, that none of the explanations yet advanced is adequate to explain the physiology of *Septobasidium*. The problems therefore are: How does *Septobasidium* get its nourishment? What is the relation between the fungus and the scale insects, and between the fungus and the 'host' plant?

To throw some light on these questions, a species of *Septobasidium*, *S. retiforme* (B. and C.) Pat., which is very

common around Chapel Hill, N.C., has been selected for intensive study, thus affording an abundant supply of readily available material for observation and experiments. Although it is not to be expected that all species would be precisely alike in physiology, yet there should be a close physiological resemblance among the entomogenous species perennially inhabiting the bark and leaves of living plants, and such I have found to be the case in a large number of other species studied.

IV. LIFE HISTORY OF *S. RETIFORME* (B. AND C.) PAT.

S. retiforme was first described as *Thelephora retiformis* by Berkeley and Curtis from material collected in Cuba. Later Patouillard (1900), recognizing the true nature of the fungus, placed it in *Septobasidium*. Coker (1920) was the first to mention the fact that scale insects were present beneath the stroma of *S. retiforme*, saying that the fungus grows on the scale insects, which are very obvious.

The fungus is very common in North and South Carolina on several species of oak, *Quercus palustris* (pin oak), *Q. phellos* (willow oak), and *Q. nigra* (water oak). It is also common in certain localities on cultivated pear, apple, and peach trees. It has been reported from several other southern states in Plant Disease Surveys and as far south as Cuba. This species is particularly common around Chapel Hill, N.C., occurring abundantly on several oaks within less than one hundred yards of the Botanical Laboratory.

S. retiforme usually attains its most luxuriant growth on the lower branches of trees, very seldom occurring on the main trunk. I have found it on limbs as thick as seven centimetres and on twigs only a year old. It usually grows on the lower surface of limbs, but old specimens may completely circle limbs (fig. 1, Pl. 15).

The fungus grows entirely superficially on the surface of the bark. I have made numerous free hand sections of fresh material, as well as stained microtome sections of material imbedded in paraffin, but have never observed any penetration, even in the outermost layers of the bark. It is very easy to demonstrate, by a simple but convincing experiment, that the

fungus is superficial. If a fresh specimen of *S. retiforme* is soaked in water for several minutes, it is possible, if the operation is carried out under water, to separate the fungus from the bark with dissecting needles, without tearing either the fungus or the bark. The bark, moreover, beneath such a specimen is usually apparently uninjured, except for a slight discoloration, and if the fungus is removed from the bark of living limbs, the bark will regain its normal colour in three or four weeks' time.

Continuous observations over a period of three years, on a large number of living specimens, have shown that the fungus growth of *S. retiforme* is perennial. Several patches of fungus growth were marked by cutting little notches in the bark beside the colony during the summer of 1927; all of these patches were still living in June 1930. As soon as it was determined that the fungus was perennial, observations were started to determine the season of the year during which the fungus grows, and its rate of annual growth. On January 18, 1929, eight patches of growth on *Q. palustris* were tagged, measured, and drawn. On this date the eight patches showed the following diameters (from 1-8): 1.5 mm.; 2 mm.; 2.5 mm.; 13 mm.; 19 mm.; 20 mm.; 15 mm.; 15 mm. On February 18, 1929, and March 1, 1929, the patches showed no increase in diameter. On April 18, 1930, the patches were again measured and showed the following diameters (from 1-8): 4 mm.; 6.5 mm.; 5 mm.; 17 mm.; 23 mm.; 20 mm., practically no growth, apparently dead; 20 mm.; 21 mm. On the patches measured, the annual increase in diameter varied from 2.5 mm. to 6 mm. It soon became apparent that the growing period occurred, as one would naturally expect, between April and November, the greater part of the growth taking place in May and June.

This species of *Septobasidium* reproduces normally only by the formation of spores. As pointed out by Coker (1920), the spore stage here is quite similar to the teleutospore and basidiospore stages in certain rust fungi. In *S. retiforme* vast numbers of resting spores are formed during the winter months near the upper surface of the fungus body. An examination of a section of the upper surface in early spring shows the resting spores or probasidia borne singly or in chains of three or

four. These probasidia show all gradations in size from the relatively few, nearly mature ones, to the vast number of young

TEXT-FIGS. 1-3.

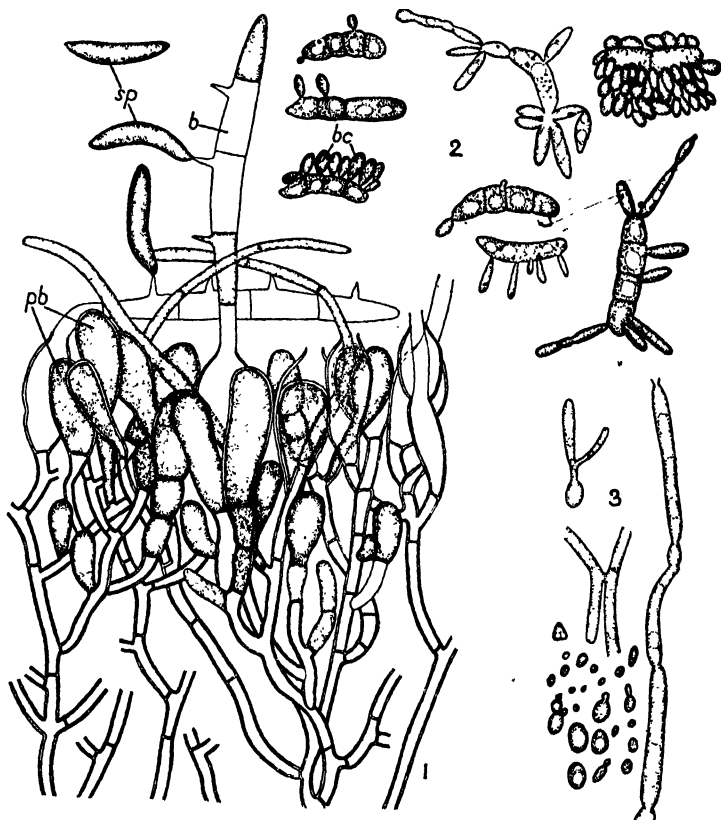


Fig. 1.—Section of hymenium or fruiting surface of *S. retiforme*. *pb*, probasidia; *b*, basidium; *sp*, spores. $\times 640$.

Fig. 2.—Budding spores of *S. retiforme* on agar. $\times 640$.

Fig. 3.—Minute bud-cells and hyphae from spores after three weeks on agar. $\times 640$.

probasidial cells, which are only slightly greater in diameter than the threads which bear them (Text-fig. 1).

When the warm, damp weather of spring sets in, the mature

probasidia germinate, forming cylindrical, four-celled basidia each cell of which usually bears one spore. Under proper conditions, each of these spores may give rise to a new plant. It is possible to collect a specimen any time between April and August, and by soaking this specimen in water and keeping it in a damp chamber for from 24–48 hours, to induce the formation of spores. In the spring or early part of the summer, the spores may be induced to form by such artificial treatment in surprisingly great numbers and for a considerable period of time. By keeping a specimen dampened, I have been able to collect large spore prints each day for nine days and could perhaps have obtained still more prints, if the specimen had not become contaminated with *Aspergillus* and *Penicillium*. In nature, however, the spores are formed only after rains while the fungus is damp; no spores at all being formed during dry weather.

Structure of *S. retiforme*.

The structure of *S. retiforme* is very peculiar and complicated, so complicated in fact that none of the previous observers has given anything like an adequate description. The plant forms oblong or circular, resupinate patches up to several centimetres in diameter. Each patch is composed of a few to many irregular concentric rings of growth, a new ring being formed each year. These concentric rings are composed of comparatively high and very irregular circular ridges between which are depressions. The outer margin of each ridge extends out into a thin, flap-like membrane which extends downward, forming a roof over the depression, and then upward, partly covering the inner side of the next ridge (Text-fig. 60). The concentric ridges are quite distinct in specimens in which the flap-like outer margin of the ridge is free; but in some specimens—for example, the one shown in the photograph (fig. 5, Pl. 15)—the concentric ridges are quite indistinct, since the outer margins of the ridges have, in most places, grown to the inner margin of the ridge next in front. There are numerous places where the flap-like margins do not grow to the ridge next in front, as shown on fig. 5, Pl. 15. but remain free, partially

closing the opening to a tunnel communicating with a chamber. In addition to the concentric ridges, and usually more distinct than these, there are also numerous anastomosing ridges and furrows which have an irregular radial direction.

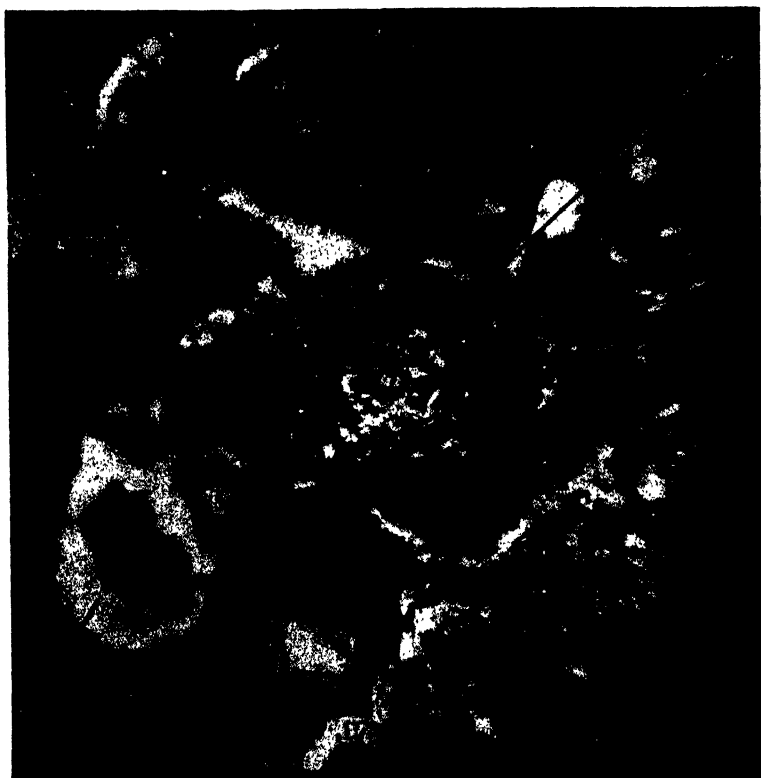
But to appreciate fully the complexity of structure, it is necessary to examine further the plant in section. In section the fungus is about 2 mm. thick in the thickest regions. It is composed of three layers: first, the subiculum, which forms a thin, closely adherent mat over the surface of the bark, and from which arise the numerous anastomosing ridges which extend in radial fashion from the centre out to the margin. The ridges take the place of the pillars found in certain other species, and give rise to, and support, the top layer. Between the ridges are tunnels, which radiate back toward the centre, and which connect with each other laterally through the depressions between the concentric ridges. The radial ridges are made up of successive series of concentric ridges, which were described above; hence a single radial ridge is composed of thick and thin regions alternating. Thus the tunnels widen out into spacious chambers where the ridges are small and low, but are narrow where the ridges are high and thick.

The floor of the chambers and tunnels is lined with a thin sheen of white hyphae; the side walls are formed by the ridges; and the roof is formed by the top layer, which varies in thickness from a fraction of a mm. up to 1.5 mm. The ceiling of the roof is composed of hyphae so closely packed that, even under a lens, the structure of the ceiling cannot be made out. The ceiling is almost black, thus differing strikingly in colour from the floor and side walls. The top layer has an exceedingly tough consistency. Many of the chambers are in direct communication with the outside through natural openings in the top layer. These natural openings are partially closed by the flap-like membranes described above (Plate 15). Other chambers, however, communicate with the exterior only through narrow tunnels.

The function of this wonderful system of chambers and tunnels immediately becomes apparent if the top layer of a fresh specimen is carefully dissected away from the supporting

ridges and folded back. Here, during the late fall, winter, and early spring months, regardless of the weather conditions, one sees within these chambers numerous living, plump, healthy

TEXT-FIG. 4.



Photograph of living, adult, healthy insect (*i*) in fungal house. Insect lies flat against bark. Fungal roof (*fr*) over insect is shown out of focus. Note tunnel, indicated by arrow, connecting chamber, occupied by insect, with exterior.

scale insects (Text-fig. 4 and Pl. 16). Each chamber usually contains only one insect, though a particularly large chamber may contain two or sometimes three. The average chamber is considerably larger than the adult insect's body. The ceiling

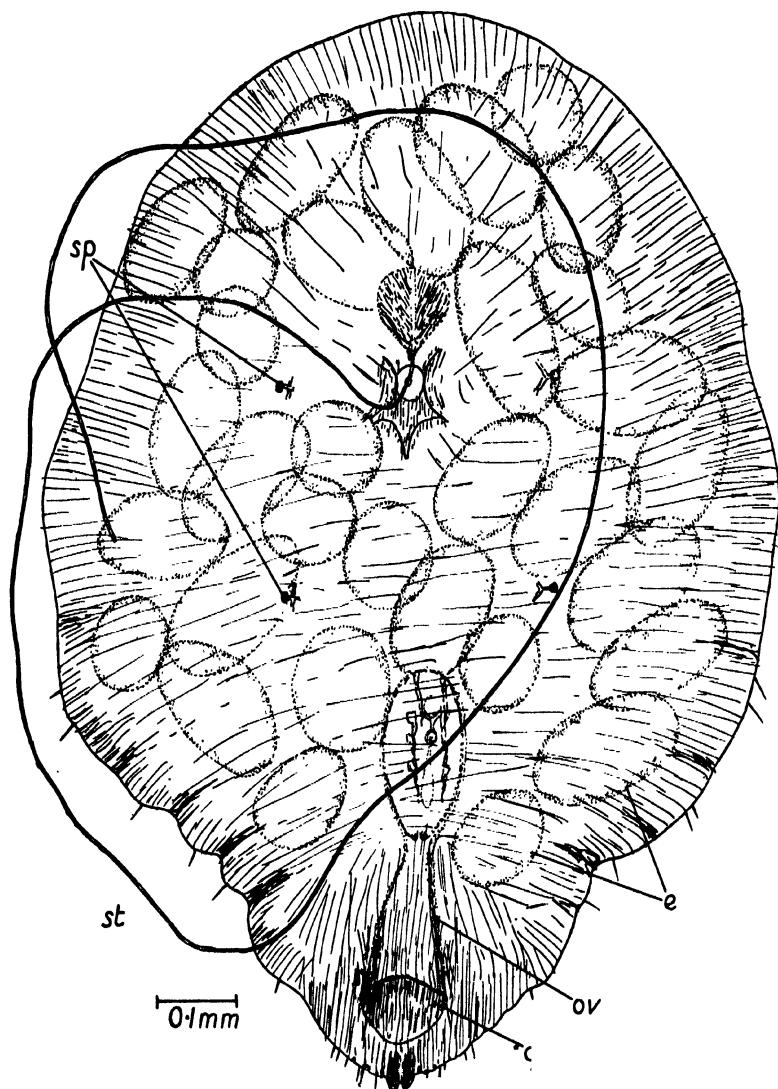
of the chamber is arched so that it does not touch the insect's body; the walls are concave, and thus adapted to the shape of the insect's sides. The floor of the chamber is the bark, and since this species does not form a ventral scale, the insect lies directly against the bark, and is not touched anywhere by the fungal threads. The fungus, however, grows over and adheres so firmly to the insect's scale (Plate 16, sc) that if the top layer of the fungus is separated from the bottom layer, the scales remain fixed to the under surface of the top layer, while the living insects remain fastened to the bark.

V. LIFE-HISTORY OF THE SCALE INSECT ASSOCIATED WITH *S. RETIFORME*.

The scale insect found beneath the fungus has been identified for me by Dr. Harold Morrison, of the U.S. Bureau of Entomology, as *Aspidiotus Osborni* New. and Ckll. Only once, out of a vast number of specimens examined, have I found a different species of insect, *Chrysomphalus obscurus*, and I found only one specimen of this. Other species of *Septobasidium*, which I have studied, may be associated with as many as three different species of scale insects at the same time, and several different species of *Septobasidium* may be associated with the same species of scale insect. I have found four species of *Septobasidium* growing on the same limb of sweet gum, all associated with *Chionaspis sylvatica*. Although the fungus *S. retiforme* seems to be regularly associated with only one species of scale, *A. Osborni*, this same scale is not limited in its fungus association to one species of fungus, but may also be found associated with *S. pseudopedicellatum*. This same species of scale may also be found growing without the fungus and may, so far as I know at present, be able to carry on its complete life-history, generation after generation, without the fungus. In this respect the scale insect resembles certain algae in Lichens.

The changes through which *A. Osborni* passes in the course of its development are very similar to those described by Comstock, for the sub-family Diaspinae, to which *Aspidiotus* belongs. The stages in the life-history of *A.*

TEXT-FIG. 5.



Adult of *A. Osborni* giving birth to young. *go*, genital opening; *ov*, oviduct; *e*, eggs; *sp*, spiracles; *st*, sucking tube. $\times 110$.

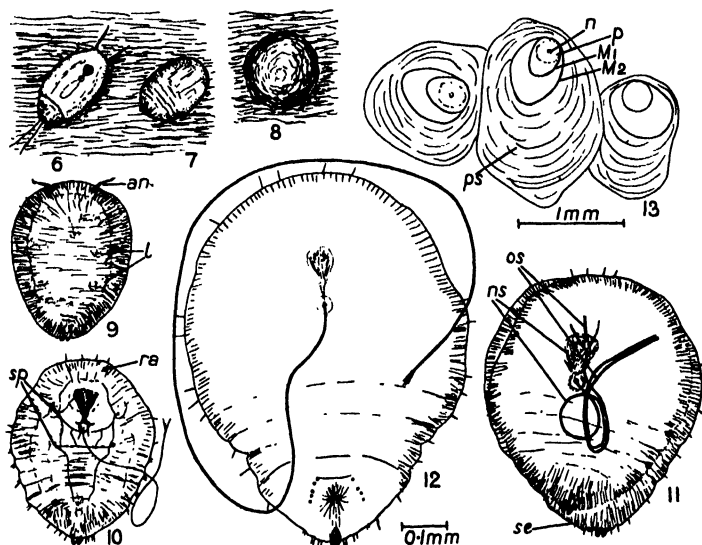
Osborni are represented by Text-figs. 5-20. The young pass down the oviduct, posterior end forward, with their three pairs of legs and antennae folded against the lower surface of their body, and covered by an exceedingly thin pellicle of material. The peculiar mouth parts, with the apparatus for sucking the juices of plants, can be seen through their almost transparent bodies. The young are born very slowly, and crawl away almost as fast as they are born, for I have never seen, in freshly collected specimens, more than a half-dozen young at the vaginal opening at one time. After remaining motionless near the opening of the vagina for from a few minutes to an hour or more, they break out of their thin pellicle and crawl away. The young, if kept in a moist chamber on filter paper, may still be crawling thirty-six hours after they have begun to crawl. In nature, however, they crawl for only a few minutes and then settle down, quickly piercing the bark with their sucking apparatus. Within a few hours the insect begins to excrete fine threads of white wax, and within twenty-four hours the body is completely covered with a thin white pellicle. This is at first round and whitish, but as development proceeds it becomes slightly eccentric and takes on a dirty greyish colour. By sticking fine 'needle-tags'¹ beside the bodies of insects which had just settled down, I have been able to follow the stages in the development of the insects as they occur in nature. About fifteen to twenty days after the larva hatches, it sheds its larval skin. With the skin are shed its legs and antennae. No trace of the legs remains, but rudiments of the antennae persist. The shed skin sticks to the under side of the thin permanent scale, becoming a part of it. The two sexes are indistinguishable up to this stage, but from now on their development differs.

The male can be recognized by the elongation of its body, while the female's body retains its more or less circular outline. The first sure sign of a male is in the appearance of two pairs of purplish pigment spots, the eye rudiments, at the anterior

¹ The needle tags were made by sticking fine needles through cardboard squares (about 0.4 mm. square). The squares were numbered so that records could be kept. I found it very convenient to use differently coloured cardboard for different lots of insects.

end of the insect. The stages in its development are shown in Text-figs. 14-20. The mature males emerge about thirty-five days after the larvae hatch. Some of the males possess fully

TEXT-FIGS. 6-13.



Camera lucida stretches all to same scale except 12 showing development of female.

Fig. 6.—Young insect (nymph) crawling on bark.

Fig. 7.—Young about two hours after it had settled down.

Fig. 8.—Young about twenty-four hours after settling down, covered with thin white waxy excretion. By this time sucking apparatus has penetrated bark.

Fig. 9.—Shed larval skin, with this are also shed antennae (*an*) and legs (*l*).

Fig. 10.—Insect in second stage, rudiments of antennae persist (*ra*) but legs are completely lost; *sp*, spiracles.

Fig. 11.—Second moult. *se*, second skin; *os*, old sucking apparatus; *ns*, new sucking apparatus.

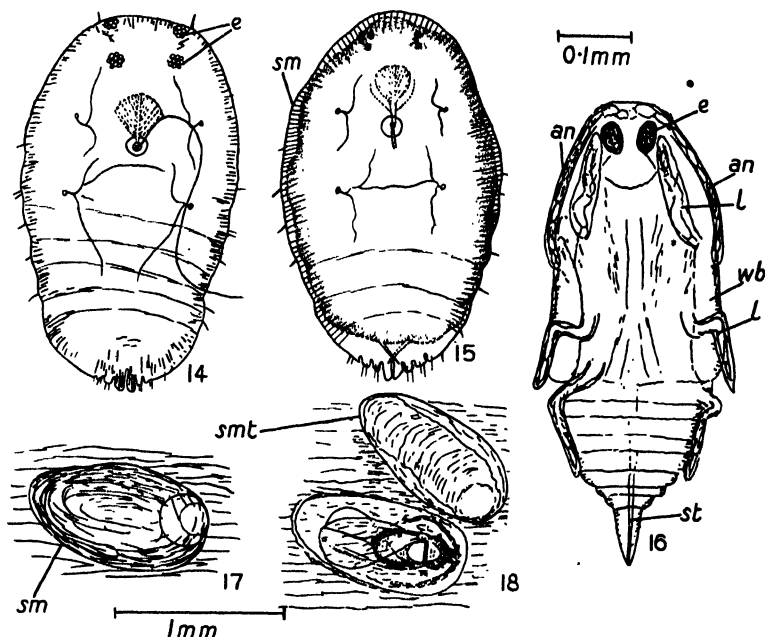
Fig. 12.—Adult female ready for fertilization.

Fig. 13.—Ventral view of three adult scales.—*p*, pellicle with (*n*) nipple like prominence; *m*₁, first skin; *m*₂, second skin; *ps*, permanent scale.

developed wings, while others show a very striking wing abnormality.

The second moult of the females takes place shortly before the males emerge. In one species in which I have observed impregnation a number of times, this event occurs shortly after the females have moulted the second time. In *A. Osborni*, I have been unable, as yet, to observe the act of impregnation.

TEXT-FIGS. 14-18.

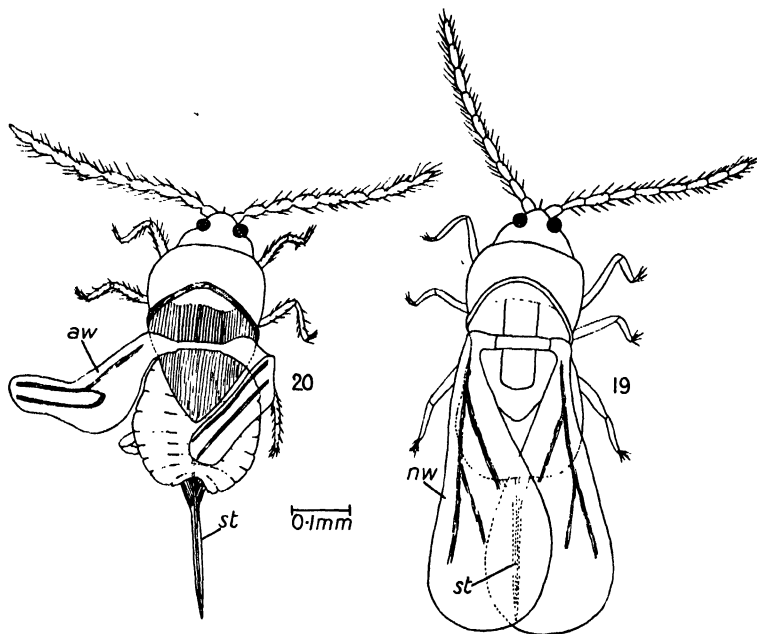


Stages in development of male of *A. Osborni* from early stage of differentiation as a male to adult. *e*, eye rudiments; *sm*, second moulted skin; *wb*, wing buds; *l*, legs; *an*, antennae; *st*, stylus; *sm*, male scale in position; *smt*, male scale turned back exposing adult male a few minutes before it was ready to emerge.

The overwintering females appear, however, to be ready for impregnation about March. At this time a most remarkable event occurs. The females, which have until now been completely hidden by the fungus, project their posterior ends up through the natural openings of the tunnels in the top layer, the entire anal segment being visible above the margin of the

fungus (Text-fig. 21). I observed this curious behaviour in a number of specimens during the early spring of 1929 and also 1930. I have also noticed, a number of times, the same behaviour on the part of overwintering females in specimens brought into the laboratory during the winter months. It is

TEXT-FIGS. 19, 20.



Camera lucida sketches of adult males of *A. Osborni*.

Fig. 19.—Form with normal wings.

Fig. 20.—Abnormal wings. Note long pointed fertilizing apparatus, *st*.

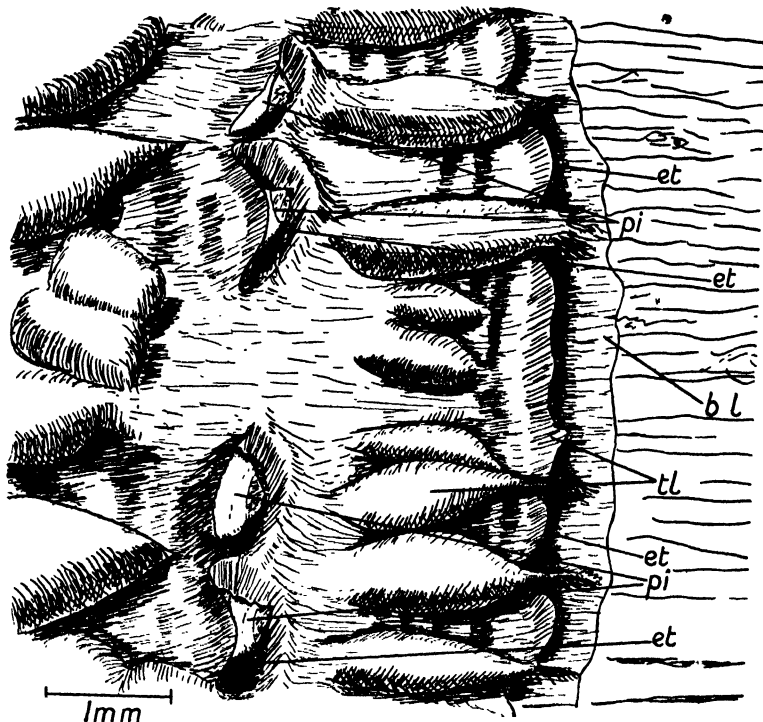
very likely that impregnation takes place while the females are in this position.

After impregnation the female's body soon becomes greatly distended with developing eggs, and in about two weeks some of the young are ready to be born. The young are usually born very slowly. A single female may continue giving birth to young over a period of from two to four weeks. In the process

of bearing young, the female's body is practically entirely used up, so that finally nothing remains but a lifeless, nearly empty cuticle.

The general relation of the scale insects to their host, the

TEXT-FIG. 21.



Sketch of part of living colony of *S. retiforme* in March, showing female insects projecting their posterior ends (*pi*) up through openings of tunnels (*et*), perhaps so that they may be impregnated by males.

structure of their sucking organs, and the general way in which they suck the plant juices, have been studied by numerous biologists. In studying the course of the sucking tube of *A. Osborni* in the oak bark, I have been unable to determine which particular tissue it seeks out. Several times in young twigs, I have been able to trace parts of the tube into the

cambium region ; usually, however, it stops before reaching this region. Oak bark, because of its hardness, is a very poor object on which to study the course of the sucking tube. In a species of tropical tree infested with *S. purpureum* and *Chrysomphalus* sp., I have found, however, an exceedingly favourable bark in which to study the course of this tube. Here the tube almost invariably penetrates to the medullary ray-cells in the cambium region (Couch, 1929). In all specimens which I have studied, the tube is surrounded by a sheath which clearly appears to be formed by the plant-cells through which the tube passes (Pl. 19). .

VI. OBSERVATIONS ON THE RELATIONSHIP BETWEEN THE FUNGUS AND SCALE INSECTS AT DIFFERENT TIMES OF THE YEAR.

1. Dormant Season.

In late fall, winter, and early spring, the fungus is in a dormant condition except for the formation of probasidia. During these months the insects too are in a semi-dormant condition. The number of overwintering insects beneath different colonies varies enormously. In colonies between 2 and 8 centimetres in diameter, the number of healthy adults is often as high as one hundred, but may be as low as a half-dozen or rarely less. Most colonies of this size have between twenty-five and fifty healthy insects (Pl. 16, *i*).

Such insects are free from fungal infection, as can be demonstrated by several methods. In the first place, the insects are nowhere in contact with the fungus which covers their bodies. If such insects are examined under the microscope *in toto*, or by crushing their bodies, or in paraffin sections, they are apparently entirely free from fungal infection. Numerous attempts to get cultures of the fungus by inoculating nutrient agar with the crushed contents of the presumably healthy insects' bodies have been unsuccessful. .

More or less embedded in the lower layer of the fungus are a considerable number of scale insects parasitized by it. These, because of their smaller size and position, are more difficult to demonstrate than the healthy insects, and can only be found

by using dissecting needles under a dissecting microscope. The number of parasitized insects is comparatively much smaller during the colder season than during the warmer months. Such insects in well-developed stages of parasitism are partly covered with a thick, dense mat of fungal threads (Pl. 19, *fm*). This mat completely overgrows the dorsal scale, extending downward, surrounding the posterior part of the insect's body. The mid- and anterior ventral regions, the regions of the mouth and spiracles, are free from the enveloping mat. The fungal threads of the mat are, of course, continuous with the threads of the main body of the fungus. The cells of the fungus mat contain two or several conspicuous globules of oil. The fungus mat may therefore be looked upon as a food reservoir for the fungus, the food being taken from the insects and stored in the mat.

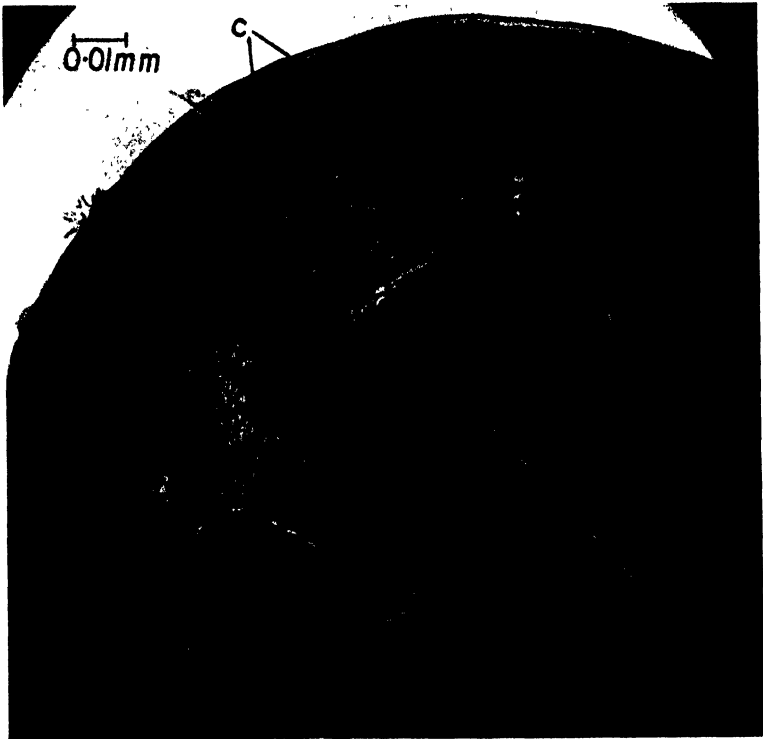
Although the fungus mat so nearly covers the insect's body, it does not become closely appressed to the latter, but is connected with the body by means of numerous, two or four times coiled hyphae (Pl. 19, *ct*; Text-figs. 40, 41, 42, 60, *ct*). These coiled hyphae give the insect's body a certain freedom of movement, thus permitting it to expand and contract—movements necessary for the insect's breathing; whereas, if the mat of fungal threads were closely appressed to the insect's body, it would soon be smothered.

These peculiar coiled hyphae connecting the fungus mat with the insect's body, are attached to the latter at the dermal pores and setae. Although the body-wall of this species of scale insect is highly elastic and relatively thin, the fungus usually does not penetrate directly through the body-wall, but passes through it at certain of the dermal pores, and the base of certain setae. I have counted as many as twelve coils attached to the insect's body at certain dermal pores and by certain setae.

By means of the threads passing through the natural apertures of the body-wall, the haustoria of the fungus within the insect's body are connected with the fungus mat, and thence with the main body of the fungus. The haustoria are found only in the circulatory system or haemocoel of the insect and are exceedingly peculiar in shape; they are in the form of coils.

The hypha in each coil or haustorium makes from about four to eight symmetrical turns, and thus each haustorium has an appearance suggesting the coil of a hot-water heater. The haustoria are sometimes in clusters of two or three, but more

TEXT-FIG. 22.



A living parasitized insect from beneath fungus showing haemocoel filled with fungal haustoria or coils (c). *sa*, suctorial apparatus. Photograph. $\times 600$.

often are connected end to end with one another by very delicate hyphae, which are only a fraction of a micron thick (Text-fig. 48). Such fine threads, in turn, connect the coiled haustoria with the main hyphae within the insect's body. These main hyphae are composed of spindle-shaped, symmetrical segments connected

end to end by the same kind of delicate hyphae as described above, so that each hypha resembles a miniature string of sausages (Text-figs. 88, 89). These hyphae are, in turn, continuous with the threads which pass through the pores in the insect's body-wall (Pl. 19, *ct*; Text-figs. 40, 41, 42, 60). Thus the connexion between the haustoria in the circulatory system of the insect and the main body of the fungus is maintained (Pl. 19).

The growth of the fungus within the insect is very slow and such parasitized insects continue to live throughout the dormant season, sucking the juices of the host plant, even after the circulatory system becomes nearly filled with the haustoria of the fungus (Pl. 19, Text-fig. 22). The bodies of the insects in which the fungus is so well developed are considerably dwarfed, and they are incapable of reproduction. During the dormant season, as we have seen, the fungus does no growing. The probasidia are formed, however, during this season and considerable food material, stored in the form of oil in the hyphal mat, is taken by the fungus from the parasitized insects. By far the most conspicuous feature of the fungal colony, provided the top layer of the fungus is removed, is the abundant, healthy, scale insects.

2. Growing Season.

With the coming of spring, the fungus starts growth as indicated by the margin changing from a brown colour to white.

The probasidia formed the preceding fall and early winter, stimulated by warmth and moisture, now begin to germinate, sending upward above the surface erect four-celled basidia, each cell of which normally bears one spore (Text-fig. 1). The probasidia do not all germinate within a few weeks, but the germination process continues throughout the spring and part of the summer, following rains. During normal spring weather, the spores are formed in greatest abundance in May. From this month on, the number of spores formed after rains diminishes until by the first of August very few or none are formed.

The spores of this species of *Septobasidium* as well as of all other species (with one exception) in which spore germinations have been obtained do not germinate directly into hyphae,

but first the spore become divided into four or rarely eight cells (two and sometimes eight in other species). Then, by a process of budding, each cell of the spore may form a number of small, oval-shaped bud-cells. If the spores are put on nutrient agar, they continue to form bud-cells for about a month before any hyphal growth can be detected with the unaided eye. If a microscopic examination of the bud-cells is made after four or five days growth, it will be seen that a few of the bud-cells have germinated into short hyphae. The bud-cells at this time vary much in size and appearance, the most usual type being the oval-shaped cells sticking together in clusters or chains resembling in a striking manner a cluster of yeast-cells. Some of the bud-cells are extremely small, being no thicker than one or two microns. The bud-cells are formed in nature, though rather sparingly, and never in such abundance as on nutrient agar (Text-figs. 2, 3).

Simultaneously with the renewed development and fruiting of the fungus, the overwintering females begin giving birth to nymphs¹ (Plate 17, *a*). During April 1929 and 1930, daily observations were made on living fungus colonies in the Arboretum to determine when the nymphs began to crawl out from beneath the fungus. In 1929 the first nymphs were seen on April 10; in 1930, on April 14. The nymphs can be discovered by looking over the surface of an infected limb with a good hand lens, and their movements can be followed in the same way. A more satisfactory method, where the supply of material is abundant, is to cut off parts of small infected limbs and study the nymphs on pieces of convenient length under the binocular dissecting microscope. Such studies have shown that the nymphs pursue either one of three courses: (1) some settle down beneath the 'maternal roof'; (2) others crawl out and settle down beneath other fungal colonies; (3) and others crawl out and settle down on bark where there is no fungal growth.

The structure of the chambers and tunnels is such that the young, which leave the maternal colony, may crawl out from the fungus through the tunnels, without crawling over the spore-bearing surface of the fungus; or they may emerge

¹ The terms 'nymph' and 'young' are used synonymously (Text-fig. 6).

directly from the maternal chamber to the spore-bearing surface, and leave the maternal colony by crawling to the margin over the top of this surface (Pl. 17, *yi, ys*; Text-fig. 60).

It has been shown by direct observations and experimentally that the nymphs are infected by the spores, or rather by the bud-cells, formed by the spores, and are very rarely or never directly infected by the fungus hyphae. This holds true equally for the young which settle down under the fungal roof on the fungal floor, as well as for the young which settle down on the bark.

The evidence for infection by the bud-cells which come from the spores is as follows. The weather during the spring of 1929 was typical for this region, i.e. warm with considerable showers every few days. A large number of young were examined both before and after they had settled down either on the bark or under the fungal roof. My records showed that over 50 per cent. of the young contained the fungus. In these early stages the fungus was in the form of bud-cells or short filaments of spindle-shaped cells held together by very delicate threads. I was unable, however, to find any spores or bud-cells of the fungus on the bodies of the insects, even though they were examined after rains when the spores were forming in large numbers. The spore-bearing season came to an end, and still I had not found any direct evidence that the spores were responsible for infection.

The lack of this evidence, and the fact that so many young, apparently healthy insects contained the fungus, demanded an examination of the possibility of congenital infection. That the fungus might be passed on from generation to generation through the egg in the form of these small, oval bud-cells was strongly suggested by the fact that, in a number of species in related genera of scale insects, symbiotic yeast-like fungi have been described, which are passed on in this manner¹ (Buchner, 1921, and others).

¹ In order to study these peculiar symbiotic fungi at first hand, I began looking for species of *Lecanium* and *Pulvinaria*, and found a species of *Lecanium* on a potted plant in the greenhouse, and Mr. Fulton, State Entomologist, sent me an excellent lot of *Pulvinaria innumabilis* (?) from Raleigh, N.C. The species of *Lecanium* was found in all

I have examined a large number of eggs of *A. Osborni* by crushing the bodies of pregnant females, but have never found any fungal cells within the eggs. I have also been unable to demonstrate the presence of fungal cells (except very rarely and then with uncertainty) in the bodies of overwintering adults, though large numbers of these have been examined at different times in toto, by crushing their bodies and by sectioning and staining. Attempts to obtain cultures of the fungus from the eggs and from the bodies of overwintering, apparently uninfected females, have been unsuccessful, although check cultures made at the same time from parasitized insects produced growth. From these observations and experiments it is obvious that the fungus is not transmitted through the egg, as is the case with the symbiotic fungus in *Lecanium* and *Pulvinaria*.

With the probability of congenital infection dismissed, the spores were again considered as the likely source of infection, and observations and experiments were renewed in the spring of 1930. By making a successful test for a spore print, it was

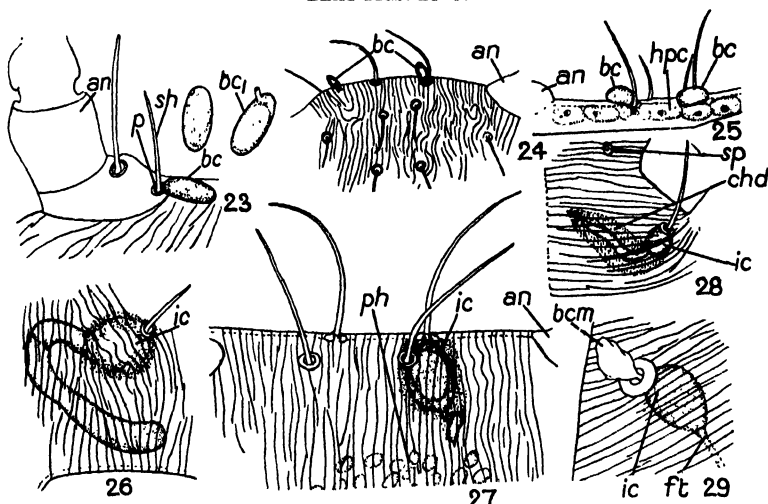
stages of development, and the elongated fungal cells were easily demonstrated by crushing the eggs and the bodies of the insects from the young crawling stage up to the mature adult stage. In young specimens mounted in water or cleared and stained in Amann's lacto-phenol-cotton blue solution, the fungus cells could be seen within the insect's body. These cells appeared to be free in the circulatory system of the insect, occupying much the same position as the early infecting cells of *Septobasidium*. The fungal cells were in the form of elongated rods, and were usually single or with a small bud-cell at one end, or rarely two cells might be held together by a short delicate connexion. In *P. innumerabilis* only the young insects were present. These contained the fungal cells in about the same numbers and position as in *Lecanium*. The fungal cells, however, were shorter and distinctly pointed at both ends, and were often joined together end to end in twos or threes by elongated delicate threads. Attempts to culture the fungus from the eggs or bodies of the insects, on maltose peptone, corn meal, carrot, malt beef-extract, potato dextrose agars and other nutrient agars, and in 1 per cent. and 5 per cent. solutions of maltose were unsuccessful. The only appearance of growth was obtained by crushing, beneath a cover glass on a maltose peptone agar plate, a large number of eggs removed from the nearly empty cuticle of a *Lecanium*'s body. Here the cells increased to about three times their usual length and then ceased further development.

found that the probasidia were ready to germinate and form spores in case a good rain provided the proper conditions of moisture. From the first week in April, however, until May 9 there was no rain. To determine when the spores were formed under natural conditions, two slides, moistened with a thin film of glycerine, were tied on the lower side of limbs just beneath a patch of *Septobasidium* on April 11. The slides were removed and examined for spores, April 12 and 13, and several times thereafter until April 25. Only two spores of *Septobasidium* were found on one of the slides, and none on the other. A similar test after a heavy rain in May gave an abundant spore print over night. The first young were seen crawling on the bark April 14. From this date on, numbers of young were seen crawling over the fungus and on the bark. Over a hundred of the young were examined for spores on their bodies, but no spores were found, and fungal cells were certainly found in only two of the insect's bodies. On May 9 and the night of May 10 there were light showers. On May 11 eighteen young were examined. All were in the first stage and had been on the bark from about two hours to two days. Only one insect showed positive signs of infection. This contained three short strings of spindle-shaped hyphae and a few bud-cells. This insect, as with many others examined in the same stage of infection, was apparently perfectly healthy and showed no external signs of infection. On each of two insects in the first stage, I found one bud-cell; and on another, which, however, was dead, there were many bud-cells, some of which had germinated, sending delicate tubes through certain of the dermal pores. This insect, though dead, gave the first suggestion as to how infection took place. On May 12 seventy-four young were taken from the bark and examined for the fungus. Some were in the second stage, but by far the greater number were in the early, first stage of development. Three of these showed very early stages of infection, but none offered any clue as to how infection took place.

Following heavy showers on May 14, ten young were collected for examination. The first one examined gave the explanation as to how infection took place (Text-fig. 27). This

insect was still crawling about over the bark when collected. Upon examination under the microscope a few minutes later, a single, large, oval bud-cell was seen just within the insect's body, attached by a delicate papilla to a fine tube which passed through the insect's body-wall by the base of one of the anterior setae. There was no external sign of an empty spore or bud-cell

TEXT-FIGS. 23-9.



Stages in natural infection of young insect by bud-cells of fungus. *an*, antenna; *bc*, bud-cell; *bcm*, bud-cell membrane; *chd*, chitinous deposit; *ft*, fine thread growing from infecting cell; *hpc*, hypodermal cells; *ic*, infecting cell; *ph*, phagocytes (?); *sp*, spiracular opening; *p*, pore at base of seta; *sh*, seta. Figs. 24, 25 $\times 700$; others $\times 980$.

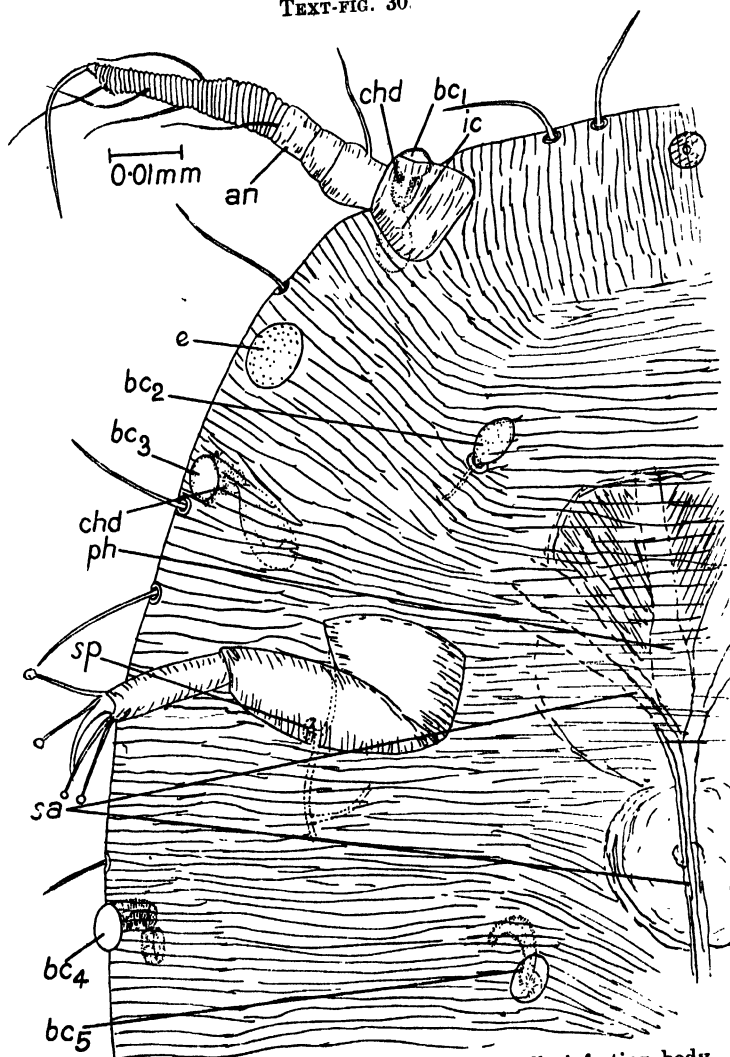
membrane. On May 15 and 16 the weather continued cloudy without rain. Twenty-eight young were examined on these two days. All but four of the insects were crawling on the bark or over the fungus. One of the four had just settled down on the bark. The fungus had entered through a pore by the mid-leg, near one of the spiracular openings. The fungus appeared, however, to be totally surrounded by a thick chitinous sheath (Text-fig. 28, *chd*). Another insect, still crawling when collected, showed an early stage of infection (Text-fig. 26, *ic*). Two other

young in the crawling stage had the bud-cells of *Septobasidium* on their bodies, one bud-cell on one (Text-fig. 23) and two on another (Text-fig. 24). The remaining twenty-four were apparently free from the fungus, both externally and internally. On May 17 six young in the crawling stage were examined, but all were apparently free from the fungus. From these observations it seemed apparent that the young were infected by the bud-cells of the fungus (Text-figs. 23-9).

To secure further evidence as to how infection took place, six young, which had just been born and were ready to begin crawling, were taken from beneath their mother and placed on the surface of a specimen of the fungus which had been soaked in water for about fifteen minutes, and kept in a damp chamber for twenty-four hours. The fungus so treated formed abundant spores, which, in turn, shortly gave rise to vast numbers of bud-cells. The insects crawl little, if at all, on a very damp surface, and therefore it was necessary, in order to be sure that they picked up spores and bud-cells on their bodies, to push them along over the surface of the fungus. Such insects, after having been on the fruiting surface of the fungus from twelve to thirty-six hours, were examined microscopically and each was infected with a few to many bud-cells. Most of the germ-tubes of the bud-cells were penetrating apparently directly through the derm; others were passing through the openings at the base of setae (Text-fig. 30). A number of bud-cells had sent germ-tubes through the insect's legs, and several germ-tubes had penetrated antennae (Text-figs. 31-6).

Infection under such abnormal conditions was usually quite different from that observed under natural conditions. The infecting bud-cells here were surrounded by a yellowish amorphous excretion (probably chitinous in nature) which appeared to glue them to the surface of the insect. Under natural conditions the infecting bud-cells are not usually surrounded by the chitinous deposit, and this fact probably accounts for the difficulty in finding external signs of infection, since the bud-cells so easily fall off after infection has taken place.

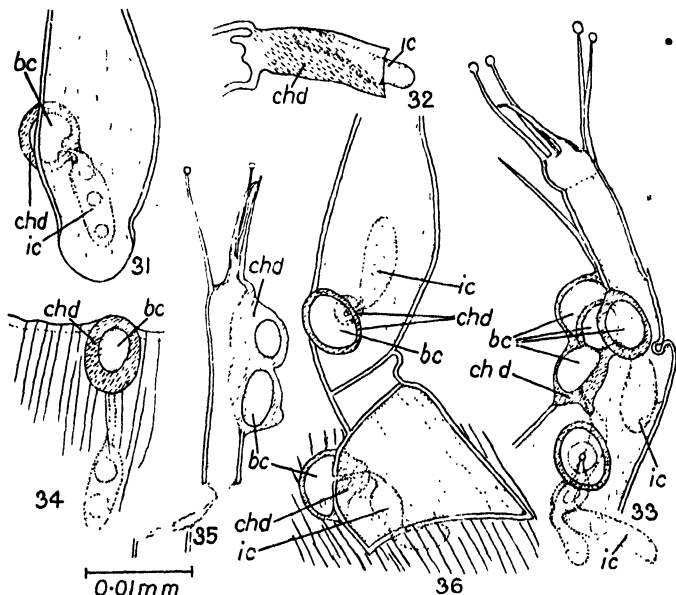
One particularly interesting phenomenon was noticed in these experiments. The legs of some of the insects were especially



Stages in artificial infection of young; bud-cells infecting body. *an*, antenna; *bc* 1-6, bud-cells; only one of which is penetrating through a natural opening (*bc*₁), others are apparently penetrating directly through insect's body-wall; *chd*, chitinous deposit; *ic*, infecting cell; *e*, eye; *ph*, pharynx; *sa*, sucking apparatus (not functioning at this time); *sp*, spiracular opening.

heavily infected, and several cases were seen where the infected part of the leg had become separated from the healthy part. Two such examples are shown in Text-figs. 32 and 35. In one of these (Text-fig. 32) the lower half of the distal segment was infected and the upper half became filled with a yellowish substance perhaps chitinous in nature. The lower part of the

TEXT-FIGS. 31-6.



Artificial infection of legs of young. *bc*, bud-cell; *chd*, chitinous deposit; *ic*, infecting cell. On fig. 32, part of leg was voluntarily amputated, infecting cell, however, remaining in stump. Fig. 35 also shows voluntary amputation of infected part.

segment was finally lost; the infecting fungal cell, however, stuck in the upper part of the segment. Two bud-cells attempted to penetrate into the other leg (Text-fig. 35), but neither succeeded. Both cells became surrounded by a very large mass of the yellowish material.

The formation of the chitinous material around the infecting spores and the autotomy of parts of the legs already infected

are without doubt efforts on the insect's part to prevent infection in the first case and to check the spread of infection in the second. Interesting in this connexion are the observations of H. P. Goodrich (1928) on a yeast disease of *Gammarus*. Goodrich found that severely infected appendages may often be thrown off autotomously.

Although these observations and experiments would suffice to show that the insects are infected by the bud-cells, further evidence showing that these are the chief, perhaps the sole, agents of infection was obtained by transferring the young, before they had had an opportunity to come in contact with the spores, to clean uninfected branches of the tree. An apparently uninfected limb on the side of the tree where there was very little infection was selected. The branches near the end of the limb, from their tips back for a distance of about three feet, were thoroughly swabbed with alcohol to remove any minute patches of *Septobasidium* and insects which might be present. A band of absorbent cotton about an inch thick and two inches wide, was tied firmly around the upper, washed part of the limb to discourage the young from crawling to the clean part of the limb, from the part which had not been cleaned. On April 28, 1930, seventy-seven young were picked up with a needle, one by one, from beneath twelve females, and transferred to the clean branches. Of the large number transferred a surprisingly small number survived, perhaps due to the excessive dryness of the season. On the second day after the young were transferred, only ten could be found. These had settled down, and excreted over their bodies the white pellicle. Only eight out of the entire seventy-seven, as far as I could find, reached the adult stage. One of these reached the stage of reproduction, giving birth to young, two of the young being found on the bark in the first stage of development. One of the eight was parasitized by the hymenopterous wasp, the latter being almost grown when examined. The remaining six died and dried up before reaching the stage of reproduction. None was parasitized by the fungus. Such an experiment as the above is defective in that the young, though several yards away from the fungus, might have become infected by bud-cells

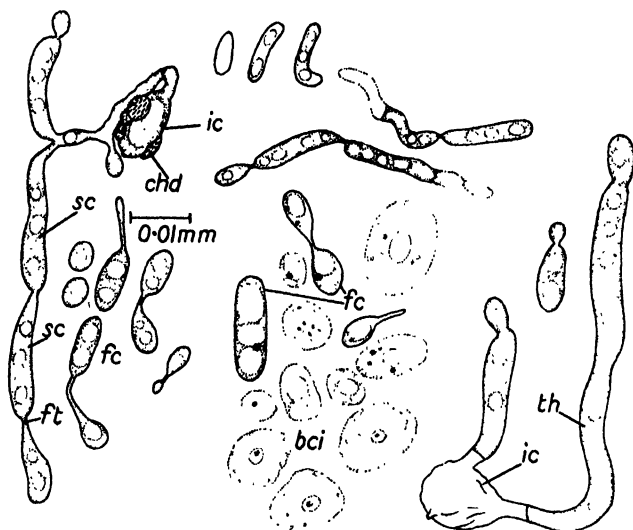
blown by the wind from fruiting patches of *Septobasidium*. The chances for such infection, however, were extremely slight, for, in addition to the fact that a very large number of bud-cells would have to be discharged into the air for one to have a chance of falling on such a small object at such a distance, the young, as the following experiment tends to show, are infected only while they are crawling.

To determine if the young which had settled down on the bark and were in the first or second stages of development could be infected by bud-cells or spores which might light on the pellicle or scale, bud-cells from a pure culture descended from spores were sprayed from a water suspension in an atomizer over the pellicles and scales of a number of young. To facilitate finding the insects the position of each on the bark was marked by sticking a needle-tag in the bark just above the insect, and the position of the insect or insects with reference to the needle was recorded. On May 3, 1930, nine young were sprayed. The same insects were again sprayed with budding sporidia on May 8. On the same date thirteen other young were marked and sprayed. Some of the young were in the early, some in the late first stage of development. The insects were examined May 26. In the first lot of nine, four had disappeared from the bark, one had dried up and four were alive and uninfected. Of the thirteen, none survived, all had either dried up or disappeared entirely. Because of the dryness of the season, these results cannot be considered as conclusive proof that the spores or bud-cells may not fall on the pellicle or scale and then grow down through either of these to infect the young insect.

Some of the insects which have become infected by crawling over the spore-bearing surface may crawl back beneath the fungus colony under which they were born, others may settle down beneath other fungus colonies, and some settle down on the bark. The first two groups of insects are solely responsible for the survival and continued growth of fungus colonies already established; and the third group which settle down on clean bark are solely responsible for the distribution of the fungus (Pl. 17, *yb 1*, *yb 2*).

A description of the fungus and insects in the later stages of parasitism was given above. An account of how this condition is brought about is now in order. The stages in the development of the parasitized insects are essentially the same as for the non-parasitized ones, except that the former usually do not

TEXT-FIG. 37.



Early stages of development of fungus in insect's body. *bci*, blood-cells; *chd*, chitinous deposit; *fc*, bud-cells of fungus; *sc*, spindle or sausage-shaped cells of fungus connected by fine threads (*ft*). Figures to left and at top $\times 830$, others $\times 1400$.

excrete a scale over their bodies, are dwarfed in size, and seldom reach the stage of reproduction. Parasitized insects usually live as long as and sometimes longer than non-parasitized insects which give birth to young.

Let us consider first the insects which settle beneath the fungus on the fungal floor. The infecting fungal cell or cells enter the circulatory system of the insect. The infecting cell is usually elliptic (Text-fig. 37, *ic*), and often may remain with one end attached to the place through which it entered. Sometimes the

infecting cell is found free floating in the insect's blood. This cell now grows into an elongated, more or less irregular thread (Text-fig. 37, *th*) which usually gives off several bud-cells (Text-fig. 37, *fc*). Sooner or later each bud-cell or thread

TEXT-FIGS. 38-40.

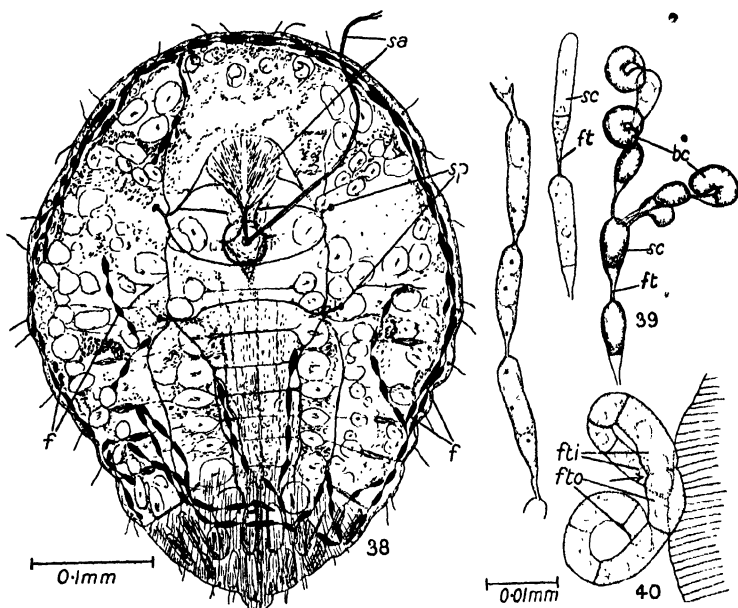


Fig. 38.—Ventral view of infected insect several weeks after infection. Insect in second stage. Fungus (*f*) is mostly in form of spindle-shaped cells held together by delicate threads. A few free fungal cells are shown. Fungus in insect's blood.

Fig. 39.—Showing spindle-shaped cells (*sc*) held together by delicate threads (*ft*) and beginning of formation of coils (*bc*).

Fig. 40.—Showing fungal hypha (*fti*) from within insect's body fusing with a fungal thread (*fto*) which has grown up over insect from fungal floor.

sprouts a fine thread which grows for a short distance and then enlarges at the end. This enlargement grows in length and diameter until a club-shaped or spindle-shaped body, pointed at the proximal end and rounded at the distal end, is formed. This new cell now sends out another fine thread, the end of which

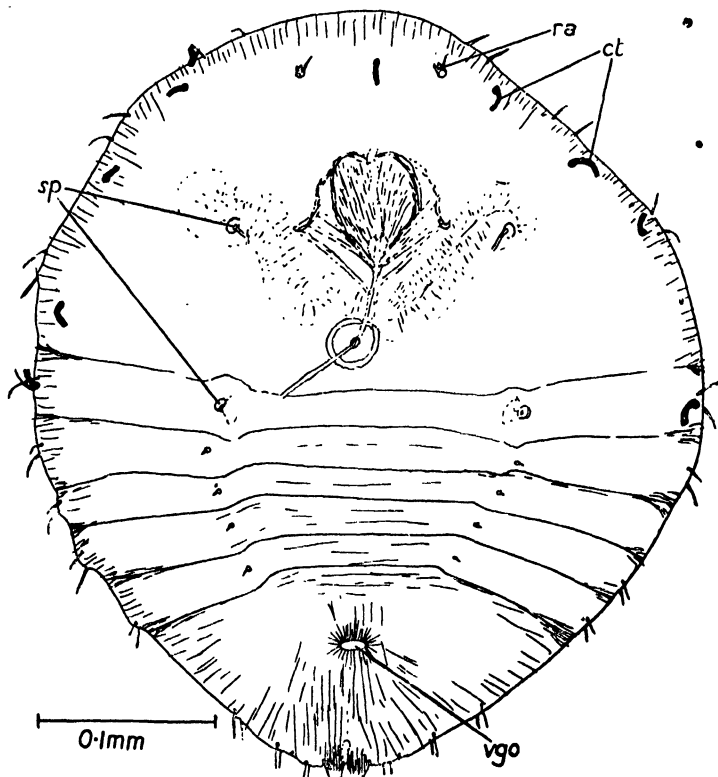
enlarges and grows into another spindle-shaped cell, and so the process may continue until chains of spindle-shaped cells connected by fine threads may be seen throughout the insect's circulatory system (Text-fig. 38, *f*). These peculiar hyphae, as suggested above, have an appearance similar to a string of sausages. They may often branch by sending out two or sometimes three fine threads from the rounded end of a spindle-shaped cell. Often the infecting cells may give rise to a number of bud-cells which may elongate and grow into the peculiar hyphae. Usually several weeks after infection and while the insect is in the second stage the coiled haustoria begin to develop (Text-fig. 39, *bc*). A coil begins to develop exactly like a spindle-shaped cell starts, i.e. a fine thread sprouts from the rounded end of a spindle-shaped cell, enlarges at the end, but instead of elongating into another straight, spindle-shaped cell it grows around in a symmetrical coil, making from four to eight turns (Text-figs. 22, 42-3, *c*). From the distal end of the coil a fine thread sprouts and another coil is formed, and so on until the coils may be in long branched series, the coils being connected by fine threads. Simultaneously with the formation of the coils, certain cells beneath some of the setae and other derm pores send fine threads through these to the outside of the insect (Text-fig. 42).

The external end of each fine thread now grows into a comparatively large, spherical cell from which sprouts a two to several times loosely coiled hypha. These coiled hyphae appear on the outside of the insect only after the second moult. By carefully dissecting out the insects in early June large numbers can be found in this stage. I have counted as many as twelve places on a single insect through which these hyphae emerge (Text-fig. 41, *ct*).

While the fungus within the infected insect's body has been developing, the hyphal threads making up the fungal floor have more or less overgrown and covered the insect's body. These hyphae, however, never penetrate into the insect. As soon as the insect moults the second time, the fungal threads which have grown over its body from the floor grow in under the moulted skin and more or less cover its lower surface. The coiled hyphae from within the insect's body anastomose with the hyphae

which grow over the under surface of the moulted skin and thus connexions are made between the fungus within the insect and the fungal threads making up the colony beneath which the

TEXT-FIG. 41.



Living parasitized scale insect dissected out from beneath fungal mat showing broken ends of threads (*ct*) connecting fungus within insect's body with fungal mat. *ra*, rudiment of antenna; *sp*, spiracles.

insect has settled down (Text-fig. 40). Thus a colony of *Septobasidium* is not a single individual descended from a single spore, but is composed of many strains, and new strains are grafted on the old ones every time the threads from an infected

insect anastomose with the threads of the fungus under which the insect has settled.

On several infected insects a very interesting condition has been found. The threads grew out through their bodies, branching until tufts of hyphae were formed, and yet there was no

TEXT-FIGS. 42-3.

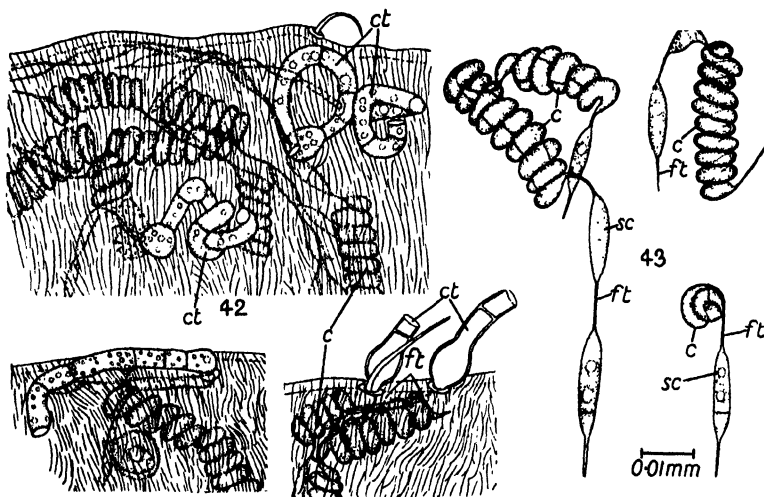


Fig. 42.—Parts of infected insects after fungus has developed considerably in their bodies showing: coiled fungal haustoria (*c*) within insect's body; delicate threads (*ft*) passing through the dermal pores connecting coils with threads (*ct*) which pass into fungal mat.

Fig. 43.—Coils from body of an infected crushed insect. (Letters as in Figs. 39, 42.)

anastomosing with the threads of the fungal floor. Such a condition strongly suggests the existence of different strains, perhaps different sexual strains, and the consequent necessity of the meeting of the complementary strains before anastomosis will take place.

Nourished by the food absorbed by the coiled haustoria in the insect's circulatory system, the hyphae covering the insect develop into a mat of threads of considerable thickness (Pl. 19). This mat of hyphae is several times thicker on the insects

parasitized during the fall and winter months than on those parasitized during the season while the fungus is growing.

The infected insects which settle down on the bark in new places are responsible for the distribution of the fungus. The young crawl about over the bark perhaps looking for some protecting surface under which they may settle down. They often crawl into small cracks in the bark, or under bits of trash adhering to the bark, or under other patches of fungal growth besides *Septobasidium*; a number, however, settle down on the bare bark, completely exposed. The greater number of these latter, whether infected by the *Septobasidium* or not, do not survive. The fungus grows very slowly in the insect's body, appearing on the outside only after the second moulting, and the insects which settle down in plain view on the bark usually do not survive to reach this stage. Usually, therefore, the young which survive until the fungus appears outside their bodies are partially or entirely hidden beneath a bit of trash of some kind and one can recognize the very minute whitish patch of fungal growth extending, over the bark, out from beneath the trash. If the trash is removed with needles under the dissecting microscope, the minute insect may be discovered more or less covered by the whitish threads. Only in a very few cases, however, have I been able to recognize the patches early enough to be certain that the fungal growth came from within the insect's body. I have examined at least a hundred such patches and each one invariably more or less covered the body of one living insect. The fungal hyphae and haustoria within the bodies of such insects were the same as were found in the parasitized insects beneath a large patch of *Septobasidium*. These minute patches of whitish growth appear early in June. By the middle of July the insect's body is entirely hidden by the fungal growth which is now about a millimetre thick though hardly more than 1 or 2 mm. wide. The fungus even at this early stage shows a differentiation into a top and bottom layer, the top layer being supported by an exceedingly thick column. The parasitized insect may be found at the base of the column surrounded, except for the mouth, spiracular, and anal regions, by the mat of hyphae. Thus the new growth of

fungus is established. It now remains for other parasitized insects to settle down beneath this colony in order for the fungus to continue growth (Pl. 17, *yc*).

By the middle of June the infected insects, which settle down in April and May beneath the fungus, have reached the adult stage and are easily recognized (Pl. 18). The number of parasitized insects at this time beneath a growing colony of *Septobasidium* is comparatively very large, and the regular arrangement of the parasitized insects between or just behind the new region of growth presents a very striking picture if the top layer of the fungus is removed (Pl. 18, *fi*). It can be shown by simple observations that the amount of fungal growth depends upon the number of parasitized insects. The number of parasitized insects beneath a colony 5 cm. in diameter was estimated at 248 by counting the parasitized ones in a quarter of the colony; another colony 1 cm. in diameter had 44 parasitized insects beneath it; another 0.5 cm., 14 parasitized; another 1 cm., 28 parasitized. The four fungal colonies showed new growth varying from 1.5 to 3 mm. in width (July 16, 1930). Six colonies were now examined which showed no new growth except in certain localized regions. Where there was no new fungal growth, no parasitized insects were present; but in the small localized regions of new growth there were one or several parasitized insects.

We may now pass to a consideration of the fate of the insects which do not become infected. Since the young, as they crawl over the damp surface of the fungus, are infected by the bud-cells, those which crawl out through the tunnels even when the bud-cells are forming, as well as those which crawl out over the top surface during dry weather, will be free from infection (Pl. 17, Text-fig. 60). As with the infected young, some settle down on the bark and others settle down beneath the fungus on the fungal floor. Of those which settle down on the bark, a surprisingly small number survive to reproduce; the vast majority perish; some dry up; others are parasitized by hymenopterous wasps, and some simply disappear, perhaps eaten by birds or other insects.

The non-parasitized young which make their home beneath

the fungus crawl in through the tunnels and settle down on the floor of the chambers and tunnels (Pl. 17, *yf*). The non-parasitized adults, however, as described above, are seated directly on the bark and are nowhere in contact with the fungus (Pl. 16, *i*). Moreover, the fungal floor which was beneath the young insect partially covers the adult. Just how this change of the insect's position in relation to the fungal floor was brought about puzzled me greatly for a long time. This past spring, daily observations have convinced me that the fungal floor is worn away beneath the insect by its movements in the formation of the permanent scale. The fine waxy threads of which this scale is made are excreted through pores on the anal segment. The scale covers the entire body, but is not fastened to the insect at any place and hence the insect is free to move about beneath the scale. That the insects do move a great deal is shown by the fact that old insects often have their spines on the anal segments almost completely worn away. By the time of the second moulting the floor beneath the insect has been almost completely worn away and fungal threads from the floor have overgrown the insect's body, so that its position can only be recognized by the pellicle which is left on top of the fungal floor, and by a slight elevation of the floor over the insect (Text-fig. 44, *p*). As the insect's body increases in size this elevation in the floor is pushed up, and soon a large part of the floor is broken off and becomes firmly fixed to the ceiling of the chamber or tunnel (Text-fig. 45, *ff*). The permanent scale formed of the two molted skins and the waxy excretion adheres to the under surface of the ceiling of the insect's chamber; while the floor of the chamber is the bark thinly covered with a whitish waxy excretion.

The insects which are born in April and May and do not become parasitized by the fungus are fertilized in the latter part of May and June and begin giving birth to young during the latter part of July. I recognized the males of this species for the first time this spring. A considerable number were found in the early stages of development, but only a few adult males were seen. The males develop as a rule on the bark, seldom being found beneath the fungus. I have never found a male parasi-

TEXT-FIG. 44.



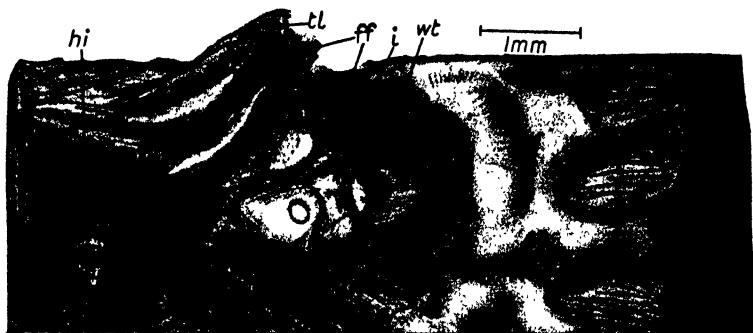
Top layer of fungus removed except in region of new growth: showing healthy insects (*i*) which settled down on top of fungal floor (*ff*) (as indicated now by pellicle (*p*) being on top of floor) now beneath fungal floor flat against bark (*b*). Note crack (*cr*) forming in floor in middle of figure; larger crack below; while floor over insect at top of figure has separated almost entirely from floor not over insect. Outlined with camera lucida July 7, 1930.

tized by the hymenopterous wasp and have seen only one parasitized by the fungus. In the latter a considerable number of sausage-shaped cells were formed, but no coils.

We have just seen that during the actively growing season of

the fungus the number of parasitized insects outnumbers several times the number of non-parasitized ones. But even with the fungus growing and forming abundant spores the insects are able to hold their own, since some of them do not become infected. The proportionate number of parasitized insects to non-parasitized ones is reversed as we pass from the actively growing back again to the dormant season.

TEXT-FIG. 45.



Showing how part of fungal floor over insect (*ff*) after separating from floor not over insect becomes attached to top layer of fungus (see also Pl. 16 showing scales attached to top layer of fungus). *ff*, fungal floor; *i*, healthy insect; *hi*, insect parasitized by hymenopterous wasp; *wt*, wax threads excreted by insect. Arrow indicates passage-way through tunnel.

3. Transition between growing and dormant season.

By the end of July, as numerous tests during three seasons have shown, the fungus has practically ceased the production of spores. During July the second generation of insects for the season are born. If the season is early, the second generation may be born as early as the latter part of June; but if the season is late, as it was this year (1930), the second generation may not be born until the latter part of July. A considerable number of the insects born during July, provided the weather conditions favour the production of bud-cells, become infected. I have found nymphs crawling on the bark, July 29, which have just been infected. It is possible that the bud-cells which remain

on the hymenial surface of the fungus may retain their vitality for some time and infect the young insects a considerable time after spore production has ceased. The bud-cells retain their vitality in artificial culture, after growth has ceased, for several weeks; but of course the damp surface of an agar plate is not comparable to the much drier surface of the *Septobasidium*.

TEXT-FIG. 46-50.

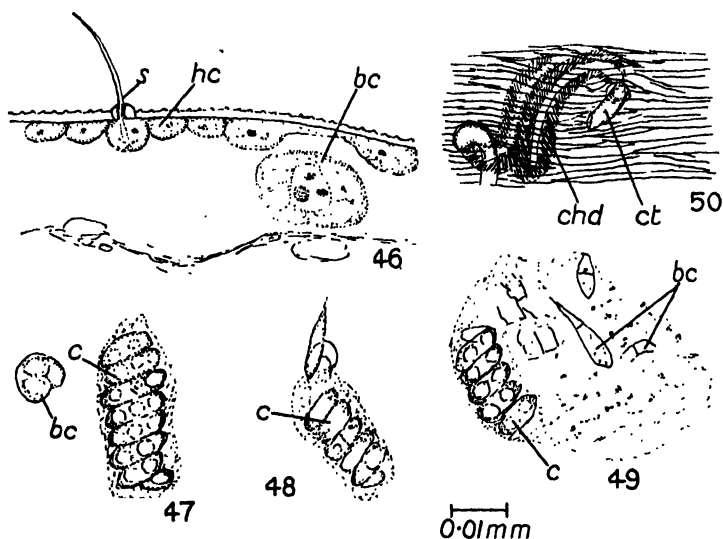


Fig. 46.—Section of healthy insect showing blood-cell (*bc*) probably phagocyte. *s*, seta; *hc*, hypodermal cells.

Figs. 47-9.—Stages in digestion of fungal coils by phagocytes. *c*, coil; *bc*, small part of coil and bits of fungal cells.

Fig. 50.—Surface view of body-wall of insect which has digested fungal cells. Note openings through which fungus penetrated are closed with chitinous (*chd*) material.

About the end of September the third generation of young are born. Exceedingly few of these become parasitized, but just how these few become infected, I am unable to say. If the fall season is late, some of the males may reach maturity, impregnate some of the females, and a fourth generation may be born in one season. The great majority of the young born in

September, however; overwinter in the adult unimpregnated condition.

Several times during late summer and fall I have observed a peculiar condition, which, although not of common enough occurrence to play an important part in the life-history of the insect or fungus, is nevertheless interesting in itself. At this time the crushed bodies of certain insects may show many of the coils surrounded by large bodies which I take to be giant phagocytes (Text-figs. 47-9). In some of the phagocytes the coils were distinct in outline, while in others the walls of the coils were hydrolized and very indistinct. In such insects the connexions between the fungal mat on the outside of the insect's body, and the coils and fungal hyphae within the insect's body are broken, and the fine hyphae which passed through the body-wall of the insect are surrounded by an amorphous chitinous deposit (Text-fig. 50).

VII. ADVANTAGES OF THE FUNGUS-INSECT ASSOCIATION TO THE FUNGUS.

Let us now pass on to a consideration of the possible benefit which the two organisms may derive from such an association as has just been described. I have never examined a colony of *S. retiforme* without finding living or the remains of once living insects beneath the stroma. Colonies of the fungus with only dead insects beneath the stroma are exceedingly rare, being outnumbered one hundred to one by colonies with living insects beneath the stroma. If a patch of fungus be found without living parasitized insects beneath the stroma, one may be sure that the fungus is either dead or in a dormant condition. Indeed, as has been shown above, the amount of fungal growth is directly dependent upon the number of parasitized insects beneath the stroma. If there is no new growth, there are no parasitized insects; if there is much new growth, then there are many parasitized insects beneath the fungus.

A simple experiment supports the conclusion drawn from the foregoing observations. Is it possible to cultivate the fungus on the bark under natural conditions without the insects? Gäumann (1922) has made extensive efforts to cultivate *S.*

bogoriense, but as he says (free translation), 'The fungus grew very poorly on twigs not infected with scale insects.' Gäumann did not have the fungus in artificial culture, but used small bits of fungus taken from a plant already established. In these experiments Gäumann was attempting to determine the number of different kinds of plants on which *S. bogoriense* would grow, and hence a large number of trees, shrubs, and succulent plants were used. The limbs or twigs inoculated with the fungus were covered with a glass tube and the ends of the tubes were stoppered with cotton wool, thus a semi-damp chamber was formed. In a number of such cultures a slight amount of growth was obtained. Growth in such chambers does not, of course, prove that *S. bogoriense* would grow in nature without scale insects.

The great difficulty in culturing *S. retiforme* from mycelium is in keeping the young, slow-growing mycelium from being overgrown by other, more rapidly growing fungi. I have made numerous efforts to establish cultures of *S. retiforme* on the limbs of trees free from insects, but without success.

A much more satisfactory means of demonstrating that the insects are necessary for the growth of the fungus in nature is to remove all traces of insects, both living and dead, from actively growing colonies of *Septobasidium*, leaving only a few small patches of the white, growing margin of the fungus. Then, by wrapping the limbs above and below each patch with a thick wide mat of absorbent cotton, young insects may be kept from crawling to the isolated fungal patches. Eight colonies of fungal growth were treated in this manner in the spring of 1929 just after growth had started, and in all cases the remaining white patches of fungus stopped growing, dried up, and eventually disappeared from the bark. In the control experiment all traces of insects were removed leaving a few small patches of the white growing margin with enough of the top layer to offer the migratory young a protected home under which to settle. In this experiment the limb was not wrapped with cotton so that the young might crawl under the small patches from near-by colonies. By the middle of June considerable new fungal growth was apparent on three of the five patches

left; and upon examination with a lens, one or more parasitized insects were found beneath each patch which showed new growth, whereas the two patches which showed no new growth had no insects beneath them.

Not only is the fungus dependent upon the insects for food, but it also depends upon the young insects for distribution. This fact brings up an interesting question. How is the fungus distributed from tree to tree? Of course there is a possibility of the bud-cells being blown from an infected to an uninfected tree and lighting on the body of a young, crawling, scale insect. A much more likely means of distribution would seem to be by birds picking up infected young on their feet and carrying them from tree to tree. However the distribution of the infected young may occur, it seems to be solely through them that new colonies of the fungus are established.

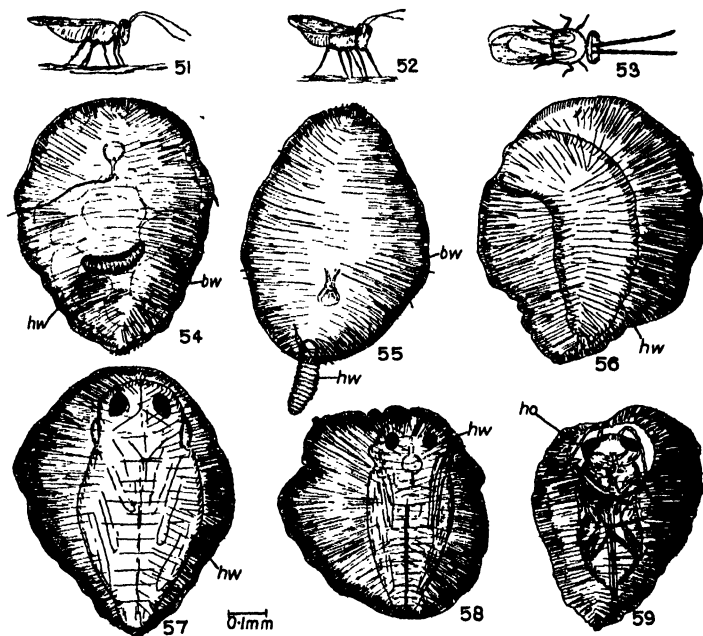
Although the fungus depends upon the parasitized insects for its food in nature, it may be separated from the insects and from the tree and cultivated on a variety of nutrient agars and in liquid media. Pure cultures have been made from single spores and from several spores, from single coils removed from the parasitized insect's body, and from slices of the plant. The fungus grows well in a great variety of media but so far no probasidia or spores have been seen in any of the artificial cultures. The failure to fruit in artificial culture may be due to the lack of the proper nourishment, furnished in nature by the scale insects; or it may be due to a complicated heterothallic condition.

VIII. ADVANTAGES OF THE ASSOCIATION TO THE INSECTS.

Whereas the fungus could not exist in nature without the insects, the insects do live without the fungus. Such insects are comparatively rare, and life for the insects which are not beneath the fungus is certainly exceedingly precarious. We do not know enough yet about the resistance of scale insects to extremes of heat, dryness, and cold; but it is certain that insects beneath the thick stroma of *S. retiforme* are ideally protected against such extremes. Among the natural enemies of scale insects are birds, and the toughness and thickness of the

fungal covering would protect the insects to a certain extent, at least, from being eaten by birds. Perhaps the most deadly of all the scale insect's enemies are certain hymenopterous wasps¹ (Pl. 17, *hw*). The female of this group punctures the body

TEXT-FIGS. 51-9.



Figs. 51-3.—Sketches of hymenopterous wasp, parasitic on *A. Osborni*.

Figs. 54-9.—Stages in the development of the hymenopterous wasp (*hw*) within the body of scale insect. Note greatly thickened, rigid body-wall (*bw*) of scale insect. Note also hole (fig. 59) (*ho*) in scale insect's cuticle chewed by the wasp through which latter will emerge.

of the female scale insect and there deposits one egg. The egg may be deposited while the scale insect is in the second, or last stage of development. Curiously enough, the development of the parasitic egg takes precedence over either the development

¹ Mr. A. B. Tahan, of the Bureau of Entomology, Washington, B.C., reports that the hymenopterous wasp parasitic on *A. Osborni* probably represents a new genus and species.

of the fungus, in case the insect is parasitized, or the development of the eggs of the scale insect, in case it has been impregnated. The egg develops into a grub which grows at the expense of the scale insect (Text-figs. 54-9). While the grub is developing, the scale insect undergoes a striking metamorphosis. The formerly, thin, elastic, partly-segmented body-wall becomes greatly thickened and rigid, loses its segmentation, and changes in colour from yellow to brick red. The grub grows until it finally occupies most of the scale insect's body. It then undergoes a slow metamorphosis into the adult winged wasp. The adult now chews a hole through the cuticle of the former scale insect's body and emerges (Text-fig. 59, *ho*). It takes just about as long for the hymenopterous wasps to go through a complete generation as for the scale insects. During the period while the young scale insects are emerging the hymenopterous wasps may be found crawling about here and there over the surface of the fungus, or rarely over the surface of the bark, searching for scale insects in which they may deposit eggs. They may also be seen between the periods when the young are born. The wasps, of course, cannot see the scale insects through the thick fungal covering, but nevertheless they are able to detect the position of their prey with uncanny accuracy. The wasp now pushes her ovipositor into the fungal covering to deposit an egg in the scale insect's body. The number of scale insects parasitized by the hymenopterous wasps varies greatly for those beneath the fungus, but may often be as high as 50 per cent.

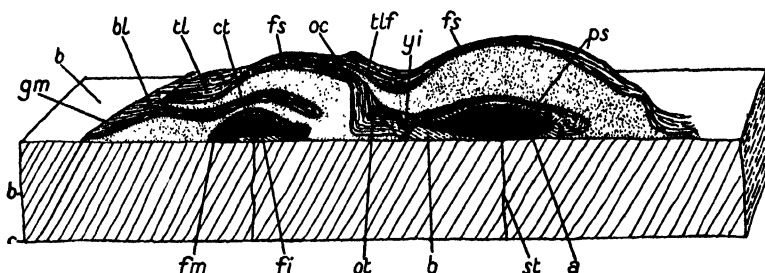
A few simple observations and measurements will show that the fungus often affords a very distinct protection to the scale insects against their most formidable enemies. The ovipositor of the wasps varies from 200 to 300 microns in length, while the thickness of the fungal roof covering the scale insects varies from 150 microns in the thinner regions (i.e. in the valleys) to over 1,000 microns in the thicker regions (the ridges). A scale insect beneath the thinner region can easily be reached by the ovipositor of the wasp; while those beneath the thicker region cannot be reached by the ovipositor. If we now examine a colony beneath which there are scale insects parasitized by the

wasps and others not so parasitized, we find the hymenopterous parasites only in the bodies of the scales over which the fungal roof is thin, whereas the scale insects whose bodies are free from the hymenopterous parasite are usually in the regions under the thick roof.

IX. NATURE OF THE ASSOCIATION BETWEEN FUNGUS AND SCALE INSECT.

Most of the previous observers have claimed that the relationship between the fungus and scale insect is one of parasitism,

TEXT-FIG. 60.



Combined surface and section diagram to show: relationship of fungus to parasitized and non-parasitized insects and relationship of fungus and insects to wood. Surface of fungus indicated by lines; section of fungus by stippling. Insects are black and bark is represented by diagonal lines. Note the suctorial tube of insects (*st*) through which insect draws its food from tree. Insect (*fi*) is parasitized by fungus and through connecting hyphae (*ct*) the fungus draws its food from insect. Insect (*a*) covered by fungus and protected by it, is not parasitized and is giving birth to young (*yi*). The young may crawl out through tunnel (*ot*) and avoid infection or they may crawl out through opening of tunnel (*oc*) over surface of fungus (*fs*), and may become infected if fungus is forming spores. *b*, bark; *bl*, bottom layer of fungus; *c*, cambium; *gm*, growing margin of fungus; *ps*, permanent scale of insect sticking to lower surface of top layer of fungus; *tlf*, flap over opening to tunnel.

that the fungus overgrows and completely destroys whole colonies of scale insects. Such a relationship may exist between certain species of *Septobasidium* and scale insects, but certainly no convincing evidence, in the way of figures or records

of continuous observations over a long period of time, has yet been produced (see historical references above). In trying to find an appropriate term to apply to the relationship between *S. retiforme* and the scale insects, it must be borne in mind that a large number of the scale insects are parasitized by the fungus, but it is clear that the term parasitism is inadequate to describe completely the relationship found here. Parasitism implies the living together of two organisms to the benefit of one and the detriment or even death of the other. The relationship found here between the fungus and insects and between both of these and the tree is represented diagrammatically in Text-fig. 60. The fungus and insects live together interdependently, the fungus furnishing a home and protection for the insects; while, in return, the insects furnish food and a means of distribution for the fungus. Some of the insects are sacrificed, but to the advantage of the insect colony as a whole; for, from the juices taken from the parasitized insects, the fungus grows and forms more houses for the non-parasitized as well as the parasitized insects. As a result of this association there is produced an entirely new type of plant. The relationship here is, therefore, obviously one of symbiosis, both fungus and insects being benefited by the association at the expense of the tree.

SUMMARY.

S. retiforme (B. and C.) Pat. is associated with *A. Osborni* New. and Ckll. The fungus insect relationship is perennial, depending only upon the life of the tree. The fungus furnishes a home and protection for the scale insects. The scale insects suck the juices of the host plant, grow, and finally reproduce their young in vast numbers. These young may pursue one of three courses: (1) they may settle down beneath the same fungus under which they were born and repeat the cycle; or (2) they may crawl out to other fungus-insect colonies; or (3) they may crawl out and settle down on the clean bark. The latter insects are entirely responsible for the dissemination of the fungus. Certain young become infected soon after they are born. The manner of infection; the development of the

fungus within the bodies of the young; and the final growth and establishment of new fungus-insect colonies are described. Parasitized insects are proportionately much more abundant during the late spring and summer months than during the fall and winter months. The type of parasitism is of a highly specialized nature. The fungus enters the circulatory system of the living insects and there develops numerous coils which absorb food from the insect. A number of the insects are finally killed and used up by the fungus; others, though infected, may digest the fungal haustoria and survive to reproduce, and some are free from any infection. Such a conjoint relationship beneficial both to the fungus and to the scale insects is obviously symbiotic.

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EXPLANATION OF PLATES.

REFERENCE LETTERS.

a, adult giving birth to young; *b*, bark; *ba*, basidium; *bl*, lower layer of fungus; *c*, coil; *ca*, cambium; *ct*, coiled thread; *et*, exit from tunnel; *ec*, empty cuticle of female scale insect; *f*, fungus; *fi*, insect parasitized by fungus; *fs*, fruiting surface, or hymenium of fungus; *fm*, fungal mat covering parasitized insect; *gl*, ganglion; *gm*, growing margin of fungus; *hi*, scale insect parasitized by hymenopterous wasp; *hw*, hymenopterous wasp; *i*, healthy scale insects; *nc*, medullary ray-cells; *mi*, mites; *ng*, new growth for 1930; *oe*, oesophagus; *ovd*, oviduct; *ot*, entrance to tunnel; *pb*, probasidia; *ph*, pharynx; *r*, ridge of fungal growth supporting top layer of fungus; *rp*, retreat pouch for sucking tube (*st*); *sc*, scale covering insect; *sh*, sheath formed by plant's cells around insect's sucking tube; *sp*, spores of fungus; *ss*, spindle-shaped fungal cells connected by fine threads within insect's body; *st*, sucking tube of insect; *slg*, salivary gland; *tl*, top layer of fungus; *yb*¹, young insect in first stage on bark; *yb*², insect in second stage on bark; *yc*, young fungal colony about one year old; *yf*, young insects settled down on fungal floor; *yi*, young insects crawling through tunnel and on bark; *ys*, young, crawling over fruiting surface of fungus; *w*, wood.

PLATE 15.

Fig. 1.—Showing a number of patches of fungus on living pin oak limb. Note narrow white margin, which is new growth, darker and wider region of last years' growth, and much darker central region of previous year's growth. Photo. April 21, 1929. \times about $\frac{1}{2}$.

Fig. 2.—A patch of growth about one year old. Beneath this growth there was one living parasitized insect. Photo. May 1929. \times about 4.

Figs. 3 and 4.—Fungal colonies three to four years old. Note white margin, the new growth. Two layered structure of fungus can be partially

made out from photographs; note openings to tunnels indicated by arrows. Photo. May 1929. \times about 4.

Fig. 5. A large fungal colony between seven and ten years old. Note white margin of new growth; irregular radial ridges and depressions between them. Note also openings to tunnels, indicated by the arrows, and flaps which partially cover openings. The concentric ridges are very indistinct in this specimen. Photo. April 21, 1929. \times about 3.

PLATE 16.

Semi-diagrammatic drawing of part of a colony of *S. retiforme* on living bark showing condition of fungus and insects in dormant season. Note fungus shows no white margin at this time of year. Top layer (*tl*) of fungus has been partly separated from bottom layer (*bl*) and rolled back showing base of ridges (*r*) which support top layer, and anastomosing tunnels and chambers in which are numerous healthy scale insects (*i*). One of scale insects (*a*) is giving birth to young (*yi*). Note particularly that healthy insects lie flat against bark, fungal floor having been worn away from beneath their bodies by movements of insects in formation of permanent scales. Note also parasitized insects (*fi*), these usually occur inbedded in fungal floor but none are here shown in this position; two mites (*mi*); scales (*sc*) adhering to the top layer; and empty cuticle (*ec*) of scale insect which has exhausted itself giving birth to young. Note tunnel indicated by arrow. Outlined with camera lucida. November 29, 1928. \times about 15.

PLATE 17.

Semi-diagrammatic drawing showing condition of fungus and insects after beginning of growing season, i.e. about last of April. This patch was about five years old. Slightly less than a quarter of patch is shown in drawing. In part shown, top layer of first and second years' fungal growth has been removed (lower right corner) leaving part of top layers of third, fourth, and fifth years' growth. Note two adults (*a*) giving birth to young (*yi*), some of which are crawling out through tunnels (*ot* and *et*), others are crawling over fruiting surface of fungus (*ys*). A number of young have settled down on fungal floor (*yf*). Note old cuticles of *Aspidiotus* (*hi*) in which hymenopterous wasps have developed. Note hymenopterous wasp (*hw*). \times about 19.

PLATE 18.

Semi-diagrammatic drawing of part of colony of *S. retiforme* near end of growing season. Top layer (*tl*) of fungus is almost entirely removed except in region of new growth (*ng*). Note conspicuous pathways leading out through tunnels, one of latter being indicated by arrow. Note four large, dark insects parasitized by hymenopterous wasps (*hi*) and three healthy insects (*i*) on bark; also numerous smaller oval outlines (*fi*). These

are scale insects of same generation as others, but parasitized by fungus. July 15, 1930. \times about 18.

PLATE 19.

Semi-diagrammatic sectional view of fungus, bark (*b*), and a scale insect (in the centre of figure) parasitized by fungus. Note sucking tube of insect (*st*) extending down through bark into cambium region (*c*). Note fungal coils (*c*) connected by very fine hyphae and spindle-shaped cells also connected by fine threads (*ss*). Some of fine threads pass through dermal pores connecting (*ct*) fungal coils (haustoria) within insect's body with enveloping fungal mat (*fm*). Note also: fruiting surface of fungus (*fs*) in which are numerous globose or ovoid cells, the probasidia (*pb*); septate basidia (*ba*), some of which are bearing spores (*sp*); young scale insect (*ys*) crawling over fruiting surface accidentally picking up spores of fungus and thus becoming infected. Between top (*tl*) and bottom layers (*bl*) of fungus is part of a chamber or tunnel (indicated by arrow).



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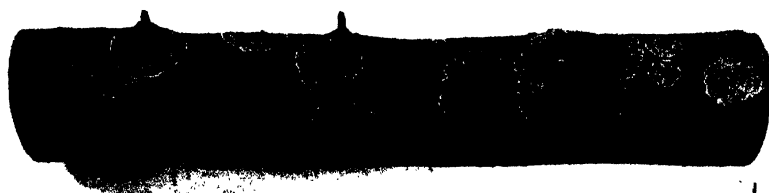
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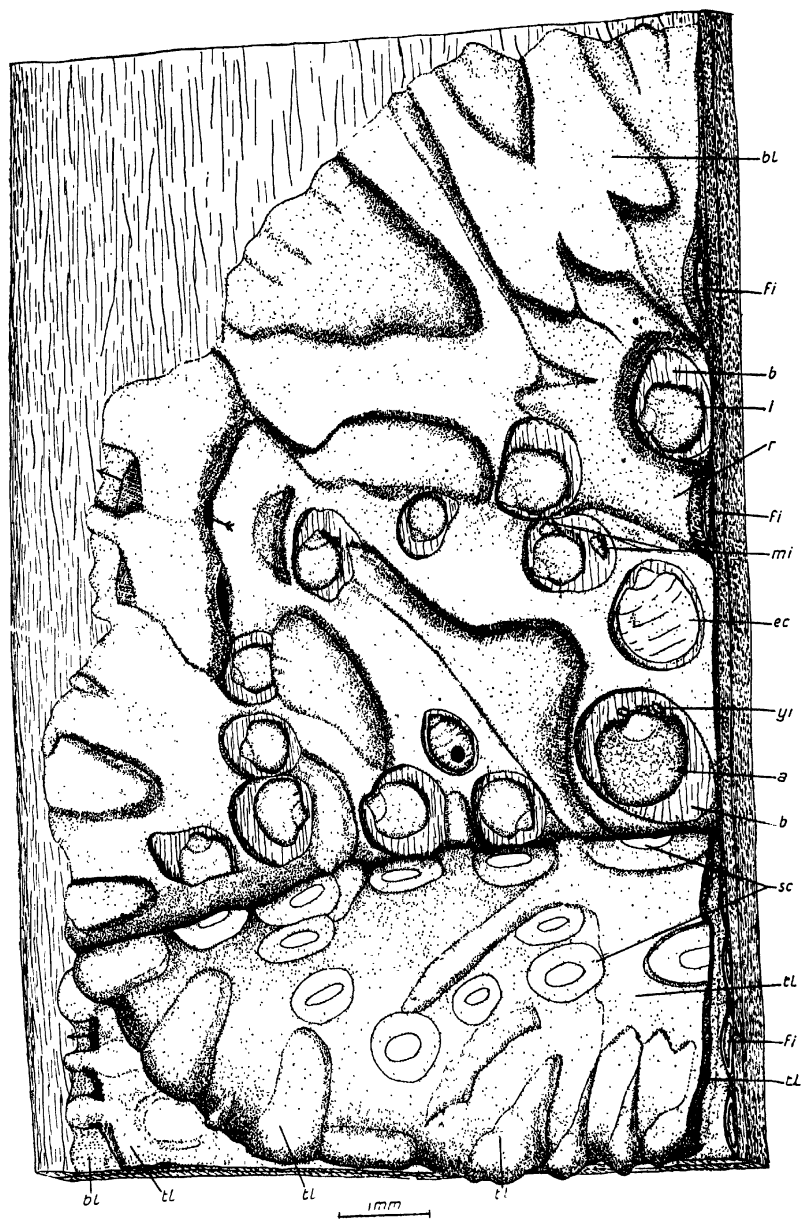
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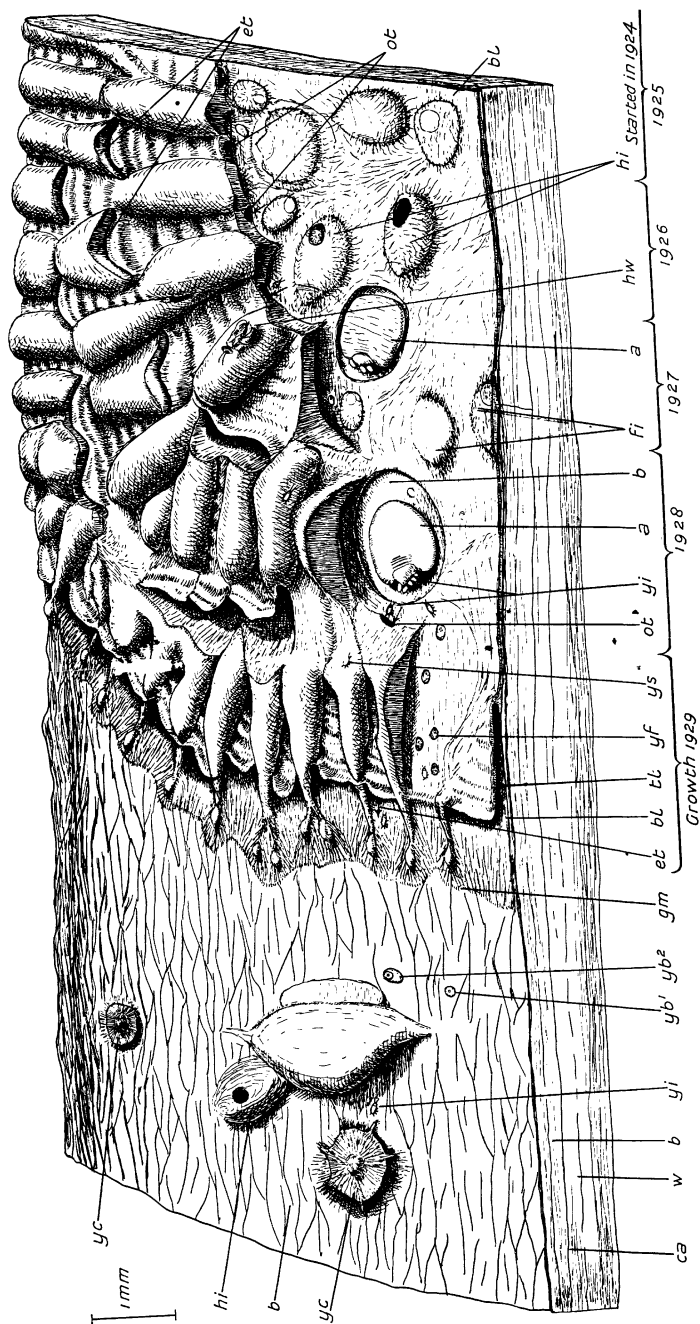


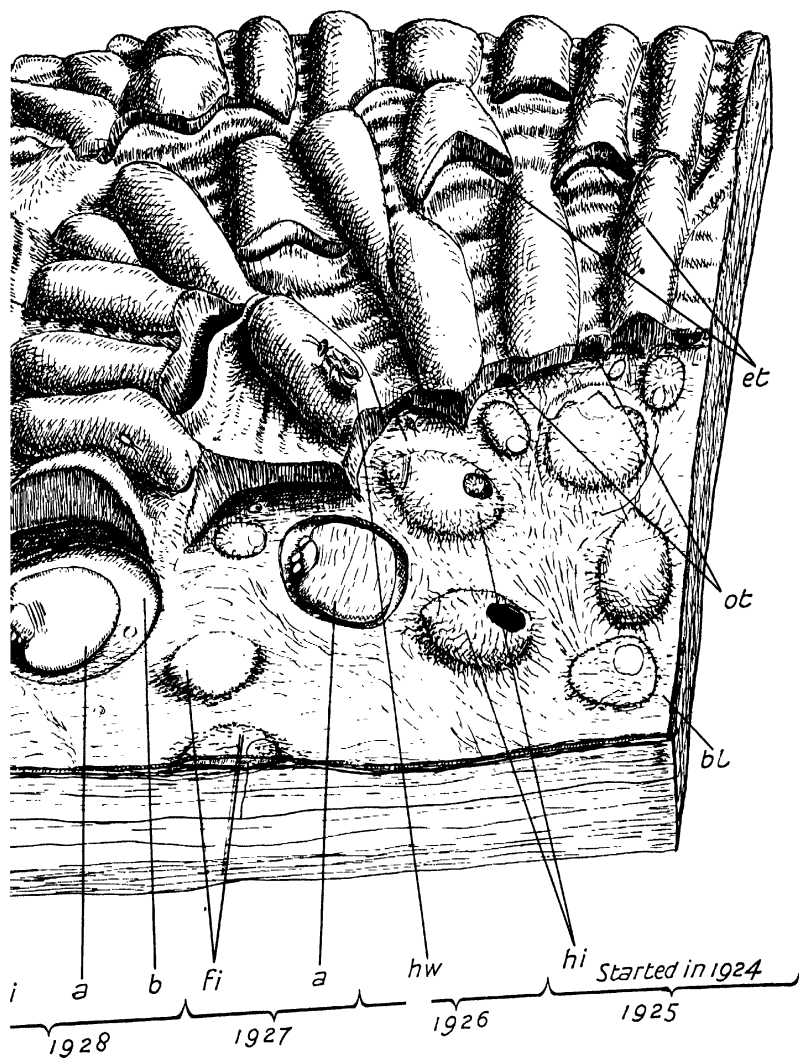
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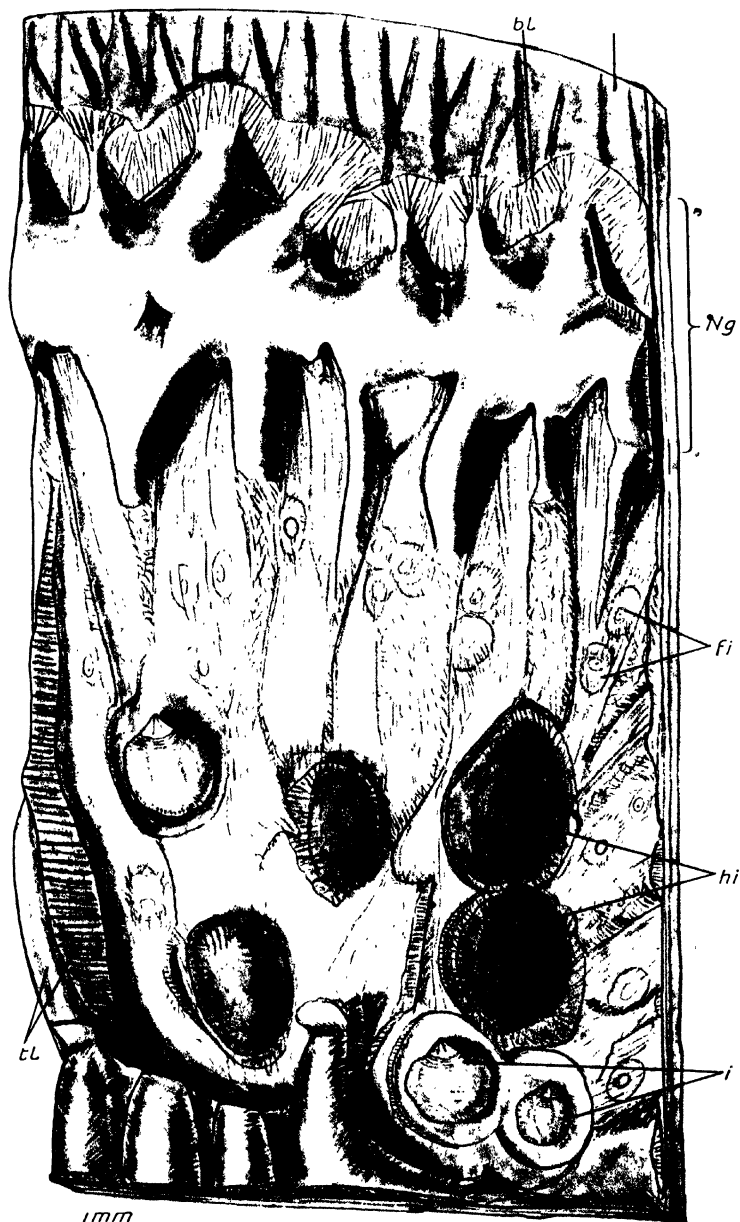


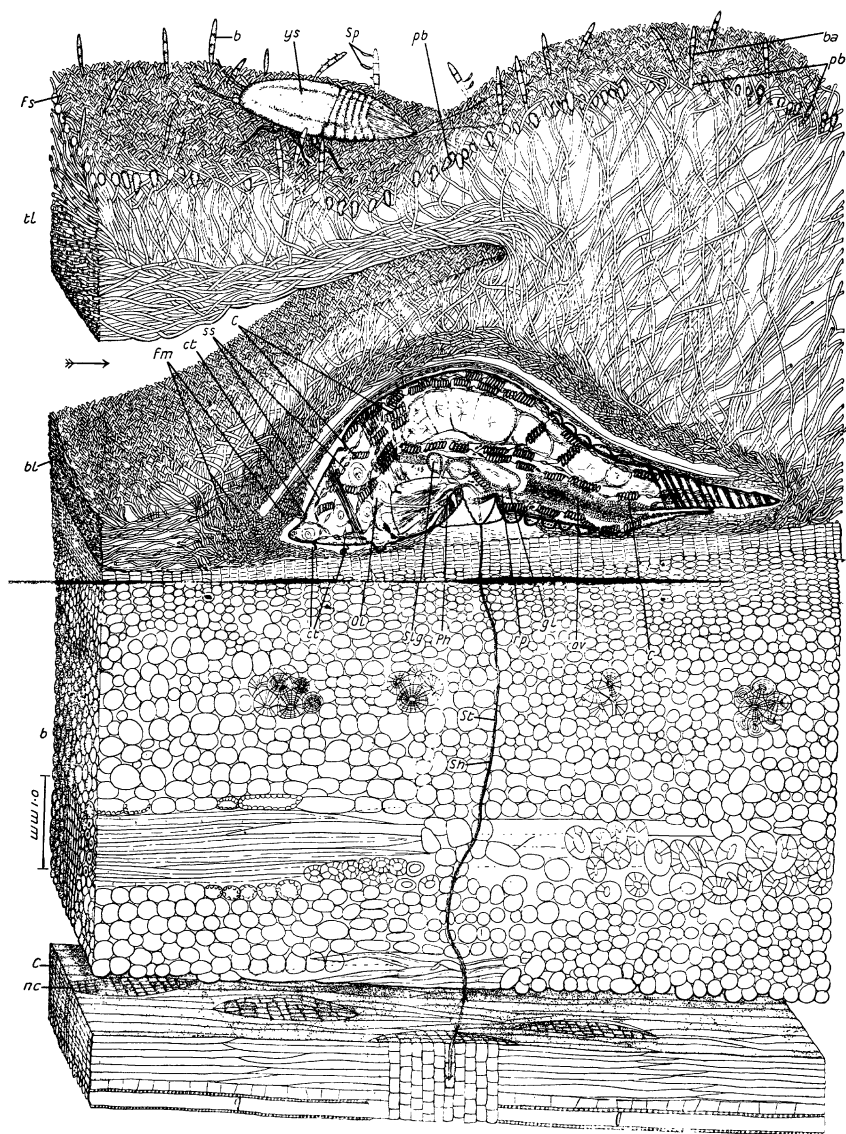
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Observations on Dicystid Gregarines from Marine Worms.

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With Plates 20 and 21.

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INTRODUCTION.

THE 'dicystid' gregarines are a little-known group of Sporozoa, and since modern technical methods have been introduced into protozoology they have scarcely been studied at all. We became interested in some that we found abundantly in certain marine worms at Plymouth; but our preliminary inquiry into the work of earlier observers revealed so many discrepancies,

and the confusion in the nomenclature appeared to be so great that we think it not amiss to prelude what we ourselves have to say with a brief summary of relevant statements that appear in the papers we have consulted.

The following definition was given by Léger in 1892. 'Les véritables dicystidées comprendront seulement deux segments: l'un intracellulaire, l'appareil de fixation: l'autre résultant du prolongement poussé en dehors de la cellule et dans lequel il n'apparaît pas de septum dans la suite: c'est l'équivalent du protomérite et du deutomérite réunis: il renferme le noyau.'

Léger took as his type a gregarine that he found in the gut of the aquatic larvae of certain dipterous insects; to this he gave the name *Schneideria*. The somewhat elaborate epimerite of *Schneideria* is shed when the organism is full-grown; the monocystid-like bodies then associate in pairs by their anterior ends, and in the resulting gametocysts spores are formed before evacuation from the host.

A year previous to the appearance of Léger's paper, Mingazzini (1891*a*) had published a note on a gregarine which he called *Ulivina elliptica*, from the intestine of the polychaete worm *Cirratulus filigerus*. He did not observe its epimerite and his drawings show the main part of the body only, in which there is no distinct septum between the anterior and posterior portions, although the endoplasm in the former is of different quality and is somewhat sharply delimited from that which lies behind it. *Ulivina elliptica* is closely related to, if not actually identical with, *Sycia opinata*, which in his paper of 1892 Léger also records from the gut of *Audouinia* (*Cirratulus*) *lamarkii*. But Léger evidently regarded *Sycia* as a tricystid. He described its development pretty fully, with a young intracellular stage, and found that gametocysts are formed within the worm's body, although he could not trace the formation of their contents beyond the sporoblast stage.¹

¹ We have frequently found the trophozoites of *Sycia* (*Ulivina*?) in *Audouinia* (*Cirratulus*) *tentaculatus* (Mont.) at Plymouth, and we consider that it is as definitely 'dicystid' as the gregarines from other marine worms that we shall describe in this paper.

In 1893 Léger noted two 'true dicystids' from the polychaete worms *Nereis cultrifera* and *Polydora agassizii*. For these he created the genus *Doliocystis*, of which he expressly states that a characteristic feature is the simple and rudimentary character of the epimerite; in *Doliocystis nereidis* it is 'un simple bouton', and in *Doliocystis polydorae* 'un tronc de cône à petite base inférieure' which 'se continue directement avec l'extrémité antérieure du second segment, allongé en forme de col'.

In 1891 Mingazzini published other papers on gregarines besides that in which he described *Ulivina*; and in one of these (1891*b*) he created the genus *Polyrabdina*, of which his account runs as follows: 'Specie dimorfe. Individui e forma di nematode piriforme. I primi hanno il corpo allungato fusiforme, e la cuticula striata longitudinalmente da rialzi numerosi finissimi.' The organisms so described were found in the intestine of the polychaete *Spio fuliginosus*, and Mingazzini considered that in its 'first form' his *Polyrabdina* was probably identical with *Gregarina spionis* recorded from that worm by Kölliker in 1845 and 1849. He slightly elaborated his description in a later paper (1893), gave some very feeble drawings, and again remarked that the second form 'which Kölliker did not see has the shape of a very small nematode'.

In 1897 Mesnil and Caullery recorded from *Spio martinensis* a gregarine which they then regarded as *Gregarina spionis* Kölliker; and in 1901 the same authors referred to a gregarine with intracellular epimerite living in *Scoelepis* (*Spio*) *fuliginosa* and 'appartenant au genre *Doliocystis* Léger'. Lühe (1904), on Mesnil and Caullery's authority, also referred to this gregarine from *Scoelepis* as a species of *Doliocystis*.

Mesnil (1907) returned to the question, and remarked, 'Il existe, je crois, chez toutes les Annélides de la famille des Spionidiens, des Grégarines aplaties, à contour ovoïde, qui sont des vraies Dicystidiées. J'ai étudié en particulier une espèce parasite de *Scoelepis fuliginosa*, à épimérite caduc, qui envoie dans la cellule épithéliale une couronne de prolongements, parfois ramifiées, sans doute amiboïdes.'

Brasil (1908 and 1909) made a further study of Léger's type-species of *Doliocystis* and of others that he had observed. In his view, these gregarines show affinities with *Lankesteria* and so lead over to the coelomic 'monocystids'. He decided that *Ophioidina Mingazzini* is synonymous with *Doliocystis*, but that Léger's name has the priority.¹ The chief contribution by Brasil to our knowledge of the life-history of *Doliocystis* is that, while the young parasite is intracellular, it later on drops out completely into the gut lumen and fixes itself to the surface of the epithelium by the epimerite, which functions as a sort of sucker. Referring to Mesnil's gregarine from *Scolecipis*, Brasil comments, 'Sont-ce là des *Doliocystis*? Fort probablement'.

By 1914, however, Caullery and Mesnil had apparently decided that the gregarine from *Scolecipis* was not a *Doliocystis*. They pointed out that Mingazzini had undoubtedly included under his *Polyrabdina* (or *Polyrhabdina*, as Caullery and Mesnil prefer to spell the name) two distinct organisms. Mingazzini's 'nematoid form' is a species of *Selenidium*, a genus founded by Giard in 1884; so that Mingazzini's 'pear-shaped form' alone should bear the name of *Polyrhabdina*. Caullery and Mesnil proceeded to give some account of this dicystid from the gut of *Scolecipis*, and they called it *Polyrhabdina spionis* (Kölliker), though it is obvious, when one reads Kölliker's papers, that the organism he observed was a 'nematoid form' and a species of *Selenidium*.² (Elsewhere, one of us (H. N. R.) has given some further description of this *Selenidium spionis* (Kölliker).) Caullery and Mesnil further noted the existence of three other species of *Polyrhabdina*—*Polyrhabdina polydora* (Léger) from *Polydora ciliata*, *Polyrhabdina brasili* Caullery and Mesnil from *Spio martinensis*, and *Poly-*

¹ Kamm (1922) considers that both *Ophioidina* and *Doliocystis* are synonymous with *Lecudina Mingazzini*, a genus founded two years earlier—1891. Bhatia (1930) agrees with Kamm.

² 'Die Bewegungen dieses Schmarotzers sind langsam und ganz deutlich; sie kommen wie bei *G. sipunculi* durch sichtbare Contraktionen und Expansionen der Leibeshöhle zu Stande' (Kölliker, 1849).

rhabdina pygospionis C. and M. from *Pygospionis seticornis*.

While studying the Protozoa in the gut of *Scolecopsis fuliginosa* and allied polychaetes at Plymouth, we have frequently come across dicystid gregarines; and since no first-hand observations on these organisms have been published since Caullery and Mesnil's brief description of *Polyrhabdina* in 1914, we think it worth while to place on record such facts as we have noted, for, as may be gathered from what has been said above, trustworthy accounts and illustrations are few, and we are persuaded that some of the earlier observations were incorrect. We have also found two dicystid gregarines in the gut of the echiurid worm, *Thalassema neptuni* Gärtner, which seem deserving of some description.

MATERIAL AND METHODS.

All the worms we examined came from Plymouth. We studied them there and also in the laboratory in London, where we were able to keep them alive and healthy for some weeks at a time. We found that only those specimens of *Thalassema* obtained by dredging were well infected; a dozen or so collected on the shore between tide-marks contained almost no parasites.

It is not easy to dissect out entire the gut of a polychaete such as *Scolecopsis*; nor, when this is done, can one see through its relatively thick wall what parasites it contains. So we examined samples of the gut-contents, and then, if it seemed to be infected, we made smears and cut sections. With the echiurid it was possible to recognize the parasites easily enough through the gut wall, so our practice here was to take out the gut entire, stretch it on a slide, and examine it under the slight pressure of a cover-slip.

We attach much importance to studying the parasites alive. There was usually enough gut-fluid obtainable for this purpose; when this had to be diluted, we found that the gregarines stood the addition of sea-water better than that of ordinary physiological salt-solution.

Smears and pieces of infected gut were fixed in Brasil's

modification of Bouin-Duboscq's fluid¹ and also in Schaudinn's fluid. Helly's fluid was occasionally used. The stains employed were Delafield's haematoxylin, Heidenhain's iron haematoxylin and Mann's methyl blue eosin. A double staining with Delafield and borax-carmin was sometimes employed with effect. Material fixed in Helly's fluid was stained in Altmann's acid fuchsin and toluidin blue. Various counterstains were employed for the haematoxylin preparations; of these, saturated solution of chromotrop 2R (1 per cent. in absolute alcohol) was found the most useful. Sections were usually cut 8-10 μ thick.

DICYSTID GREGARINES FROM TWO POLYCHAETE WORMS.

A. *Polyrhabdina spionis* Ming. n.var. *bifurcata* from *Scolecopsis fuliginosa* Clpdc.

Eighty-five of the two hundred and fifty specimens of *Scolecopsis fuliginosa* examined by us contained a cephaline gregarine that agrees in many respects with Caullery and Mesnil's description of *Polyrhabdina spionis*.

The organism (fig. 1, Pl. 20) lives in the middle portion of the gut, and is almost always attached to the wall by its epimerite. This is a very definite, transparent, knob-shaped structure, with a circle of fourteen to sixteen minute teeth at its base, and, as its crown, two much larger, diverging, claw-like processes, which are flattened towards their tips (fig. 4, Pl. 20). Caullery and Mesnil figure these processes, or 'barbelures', as at least four in number, sometimes branched; and in one drawing, where the epimerite is seen in end-on view, as many as nine are shown. Moreover, according to these authors, the whole epimerite is intracellular and the processes are said to be amoeboid structures which draw nourishment from the host-cell. We have examined many sections, but have never found the epimeritic claws lying within the substance of the cell; they are merely applied to the surface of the epithelium,

¹ Sat. solution of picric acid in 90 per cent. alcohol	2 parts
Sat. solution of corrosive sublimate in distilled water	3 "
Formalin	1 "
Glacial acetic acid	2 "

sometimes embracing more than one cell. There is no evidence at all that they are amoeboid or capable of absorbing food; we never find that they are perforated at their tips, and they seem indeed to be cuticular anchors, comparable with the epimeritic outgrowths of gregarines like *Nina* (*Pteroccephalus*). The whole surface of the epimerite stains black with iron haematoxylin and pink with Mann's methyl blue eosin.

We have found it difficult to decide whether the differences in the structure of the dicystid gregarines observed by us and by Caullery and Mesnil from the same worm (though admittedly from different localities) are such as to justify the creation of a new species. On the whole, we think it best to regard the Plymouth type as a new variety, *bifurcata*, of *Polyrhabdina spionis* Mingazzini.

Behind the epimerite the main mass of the body of a full-grown gregarine is a sac-like thing measuring about $180\mu \times 36\mu$; it is always circular in cross-section (fig. 8, Pl. 20)—not flattened, as Mesnil seems to suggest that these dicystid gregarines of polychaetes are wont to be. There is a thick pellicle, which is longitudinally grooved. The pellicle is very much thinner at the bottom of the grooves, and it seems probable that fluid nourishment from the worm's gut can be absorbed along these lines. The ridges between the grooves stain deeply with iron haematoxylin, and each contains three to five siderophilous fibres longitudinally disposed. (Caullery and Mesnil make no reference to these characteristic striations.) The ridges converge towards the anterior and posterior ends of the deutomerite and may actually become concrescent. The gregarine appears quite immobile when alive, and there is no evidence that the fibrils on the ridges are myonemes. We do find, however, that a layer of circularly disposed fibrils lies beneath the longitudinal grooves; and the variation in width of the grooves and the seeming breadth of the dark, fibrillar ridges between them may be affected by the contraction of these elements.

The endoplasm in the main portion of the body appears dark and granular when alive, but clear and alveolar in fixed specimens, except where parasites are present. As in *Sycia*

(*Ulivina*) the endoplasm is more transparent just behind the epimerite, and there is sometimes a fairly definite line of demarcation between this clearer region and the more granular endoplasm behind, though there is no actual septum. The nucleus measures $10.5\mu \times 7.5\mu$ in a full-grown specimen; it lies in the middle of the body, midway between the poles of the main segment (fig. 1, Pl. 20); in parasitized gregarines it gets crushed to one side and stains more faintly than normal. The nuclear sap is filled with fine, evenly disposed granules of chromatin; usually two karyosomes are present; one is fairly large and stains less intensely than the other, which is always a small, densely siderophilous granule. Both stain red with Mann's stain.

Caullery and Mesnil maintain that, in the early part of its life, *Polyrhabdina spionis* lives mainly within the host-cell. This is what one would expect to find. They give drawings to show the entry of the anterior end of the young, undifferentiated organism into the cell, and its growth there. The youngest stages we have found in *Scolecopsis* from Plymouth measure about $7.5\mu \times 8\mu$. These are clinging to the surface of the cells by means of the small teeth at the base of the epimerite; the characteristic anchors must be developed later. In other respects these young gregarines are just like the adults, but on a small scale. We have no evidence that the organism is ever even partially intracellular. Caullery and Mesnil's pictures of that stage suggest that they may have confused with *Polyrhabdina* a young intracellular *Selenidium*, two species of which are its common accompaniments in *Scolecopsis*. One of us has described these 'schizogregarines' elsewhere (Ray, 1930*a*). We are the more inclined to think that Caullery and Mesnil were mistaken, since they undoubtedly confused *Selenidium* with intracellular stages of a coccidian-like sporozoan that is also common in the same situation (Ray, 1930*b*).

Our impression then is that the intracellular stage of *Polyrhabdina spionis*, if it occurs at all, must be very brief, and that during almost the whole of its life this gregarine is extracellular, clinging to the epithelial surface by means of its

epimeritic anchor. The cells to which it hangs rarely show hypertrophy.

We are unable to say anything further concerning the life-history. Where the infection is heavy, these relatively large organisms lie so close to one another in the narrow lumen of the host's gut that association might easily take place without any relaxation of the grip upon the epithelial cells; but we have never seen such a thing happen, though we have searched long and carefully. Nor have we ever found spores, either within the worms or in the slime mixed with faecal débris that collects round these in the aquarium.

We have often seen the curious parasites of *Polyrhabdina* that Caullery and Mesnil (1897 and 1914) named *Metchnikovella*; but we are as little able as were these authors to decide what is the true nature of these organisms. We have nothing to add to the description given by Caullery and Mesnil of *Metchnikovella*, and we suppose that the species we find is *Metchnikovella spionis* Caullery and Mesnil, although theirs was from *Polyrhabdina brasili* in *Spio Martiniensis* and the cysts in our specimens sometimes exceed the dimensions they give. On Pl. 20 are three drawings (figs. 5, 6, and 7) of this parasite as we have seen it in the *Polyrhabdina* from *Scolecopsis fuliginosa*.

B. *Polyrhabdina polydorae* Caul. and Mesn.(?) from *Polydora flava* Clpde. (= *Lecudina polydorae* Kamm 1922).

As already stated (p. 441), Léger (1893) described a dicystid gregarine from the intestine of the polychaete *Polydora agassizii* and he named this *Doliocystis polydorae*. It was said to have a very simple epimerite, 'un tronc de cône à petite base inférieure'. Brasil (1909) when working on other species of *Doliocystis*, referred to *Doliocystis polydorae* Léger, said that he had not actually seen it, and then went on to observe that the genus *Doliocystis* seems to have much in common with *Lankesteria*. It is rather surprising, therefore, that Brasil should consider as 'probably a *Doliocystis*' the dicystid with ramified epimerite recorded by Mesnil (1907)

from *Scolecopsis fuliginosa*—a near relative, that is to say, of the gregarine which we have just described above.

Caullery and Mesnil merely added to the confusion. They remarked (1914a) that the species of *Polyrhabdina* (*Polyrhabdina brasili*) which they find in *Spio martinensis* has an epimerite of fundamentally the same type as that of *Polyrhabdina spionis*, and they proceed, 'Il en est sans doute de même de l'épimérite du *Doliocystis polydora* décrit par Léger; nous avons pu le vérifier pour une Grégarine de *Polydora ciliata*.' And in the list of species of *Polyrhabdina* at the end of their paper they insert 'P. *polydora* (Léger) de *Polydora ciliata*'.¹ Caullery and Mesnil seem to agree with Brasil that *Doliocystis* is related to the monocystid gregarines; and it is not clear how they square this with their assertion that *Doliocystis polydora* Léger is a species of *Polyrhabdina*. Kamm (1922) apparently follows Caullery and Mesnil in regarding the gregarine as a *Doliocystis*. She refers to it as *Lecudine polydora*.

Léger's accounts of what he observed should always be taken seriously. It is unlikely that so careful an observer would have overlooked the somewhat complicated structure that one would suppose, from Caullery and Mesnil's own statement, the epimerite of a *Polyrhabdina* should show.

We have not seen *Polydora ciliata*, nor its variety *agassizii*, but we have frequently found in the intestine of *Polydora flava* at Plymouth a dicystid gregarine undoubtedly related to the *Polyrhabdina* from *Scolecopsis*. This may be the species observed by Caullery and Mesnil, and we have therefore given their names as the 'authority' for *Polyrhabdina polydora*, for there is no good reason to suppose that Léger ever saw this organism.

We say that the dicystid from *Polydora flava* may be Caullery and Mesnil's species, but these authors give no real description of what they saw. We think it worth while to publish a few notes and drawings, although unfortunately we are not able to give any account of the life-history. We examined many worms, and though 75 per cent. were infected, often very

¹ *Polydora agassizii* is a variety of *Polydora ciliata*.

richly, we always found the gregarines well developed but apparently not yet ripe for association.

As we have found them, the trophozoites measure $100\text{--}180\mu \times 30\text{--}40\mu$ (fig. 8, Pl. 20); we have never seen any young forms. The pellicular layer is relatively thinner than in *Polyrhadinaspionis*, but carries comparable, though slighter, longitudinal striations (each made up of a number of dark-staining fibrils) with thinner-walled grooves between. The nucleus measures 15μ across and lies in the middle of the sac-like body; there may be more than one karyosome, and one of these frequently shows a lighter core. The chromatin is less evenly disposed in the nuclear sap than in the last species; it often forms small clumps of irregular shape.

The epimerite has no claws at its tip; but twenty-two to twenty-four slender, dark-staining prongs arise in a circle slightly anterior to a collar of similar material at the base (fig. 9, Pl. 20), and are seemingly the counterpart of the small denticles in that position on the epimerite of *Polydora spionis*. By means of its epimerite the gregarine clings closely to an epithelial cell; but, in the adult condition anyhow, it always remains quite external thereto. The delicate prongs from the collar arch over and hold on to the surface of the cell, and the knob-like portion in front of them bulges into a depression in it. The affected cell tends to increase considerably in thickness, but does not elongate perceptibly nor project beyond its fellows. There is no evidence that such hypertrophy as occurs is caused by other than purely mechanical interference by the parasite, for the prongs of the epimerite have no aperture at their tips, nor does the knob really enter the cell anywhere. As in *Polydora spionis*, the epimerite seems to persist throughout the gregarine's growth period; but as we have seen no young forms, we have no evidence as to how it develops.

A species of *Metchnikovella* attacks this gregarine also, and, as it is not like any of the seven described by Caullery and Mesnil, we propose to call it *Metchnikovella caulleryi* n.sp. Portions of the hyphal stages are shown in figs. 11 and 12 (Pl. 20). The tube-like 'cysts' measure $85\text{--}100\mu \times 2.5\text{--}3\mu$ (fig. 13a, Pl. 20); they scarcely taper at all towards their ends,

and these contain very little of the dark-staining plugging material that is so obvious in *Metchnikovella spionis* (fig. 13 b, Pl. 20). The 'spores' measure about $3\mu \times .75\mu$ (fig. 13 c, Pl. 20): there are 20 to 30 within each cyst. In some of the infected gregarines in smears, we find that the epimerite has been torn away, and the cysts of the *Metchnikovella* are seen escaping, as they might from a bottle of which the stopper had been removed. It seems improbable that this is their normal mode of emergence. Perhaps the host gregarines disintegrate and deposit their contents in the gut; we have never found completely free cysts, and we have often seen the spores escaping from the cysts into the impoverished endoplasm of a heavily infected gregarine.

DICYSTID GREGARINES FROM *THALASSEMA NEPTUNI*
GÄRTNER

The gregarines we have described above are from the gut of polychaete worms; and this seems to be the habitat of most of the species known.

The following notes refer to two dicystids that we find very frequently in the intestine of an echiurid worm at Plymouth, *Thalassema neptuni* Gärtner, where they occur in company with the holotrichous ciliate *Ptyssostoma thalassemae* Hentschel, and a peculiar acephaline gregarine which we describe elsewhere.¹

We are reluctant to give new generic names to these organisms, for it is obvious that, until much more is known about the life-cycle of the dicystid gregarines, it is futile to express an opinion as to their relationships or to decide which of their morphological characters is to be considered as of 'generic value'. With the tricystid gregarines, the structure of the spore seems to be the best criterion of relationship; and, next to that, the form of the epimerite. For reasons that will appear later, we can say nothing definite about the spores of the gregarines from *Thalassema*.

¹ In 1881 Lankester described a 'gregarine' from *Thalassema neptuni*, but this was found in the egg-sacs of a female, where it had invaded the developing eggs. We have seen this organism, and have recently stated (1929) that it is a coccidian.

Their epimerites are complicated structures which seem to anchor them to the gut-wall throughout their growth as trophozoites; this rules out our placing them in the genus *Doliocystis*. We have seen *Anchorina sagittata* Leuck. from the gut of the polychaete *Capitella capitata*, a dicystid which, in spite of its peculiar form, has points of similarity to *Doliocystis*; it has almost nothing in common with the gregarines from *Thalassema*. We have carefully examined *Sycia* (*Ulivina*?) from *Audouinia*, and we regard it as something quite distinct from our parasites. Their epimeritic structure is unlike that of *Polyrhabdina*, described above. Among well-authenticated genera there remains only *Schneideria*; but this dicystid is parasitic in aquatic insect larvae, and its spores develop while the gametocysts are still within the body of the host. Whereas, so far as we can judge, the associated gametocytes of the gregarines from *Thalassema* do not develop further until they have reached the water outside.

We therefore feel obliged to create two new genera, *Hentschelia* and *Lecythion*, to include the dicystid gregarines from the echiurid.

A. *Hentschelia thalassemae* n.gen., n.sp.

The trophozoites are most abundant in the middle portion of the gut, i.e. the part of the intestine that is accompanied by the collateral tube; in the narrower, thick-walled region they may be so abundant as almost to block the lumen. Associating forms occur further back and are found in the rectum chiefly, whence they are evacuated with the faecal balls.

We have found a few young stages. These lie within the epithelial cells (fig. 14, Pl. 20). The smallest we have noted measure about $15-22\mu \times 28\mu$. Already the inner end of the parasite is partially differentiated as the epimerite; the nucleus is relatively enormous. The host-cell is considerably enlarged and has paler contents than have its neighbours, and even at this early stage it tends to get dragged up from the rest of the epithelium, to which it remains attached by rooting processes of cytoplasm. As in cephaline gregarines generally, it would

appear that, as the organism increases in length, its posterior segment breaks through into the gut and hangs free there, while continuing to grow to full size; the epimerite, however, always remains lodged within the host-cell, and so firmly that, when one makes a smear, the two usually come away together.

Including its epimerite, the full-grown trophozoite (fig. 15, Pl. 20), measures $75-98\mu$ in length; the greatest width of such forms is $30-45\mu$. The posterior end is often pointed like the free end of a lemon. But the deutomeritic segment varies very much in relative length and breadth from specimen to specimen; and this suggests that there may be a layer of circularly disposed myonemes below the pellicle though we have never observed any contraction, and the gregarines remain quite inert when detached from their hold. The pellicle is very thin compared with that of *Polyrhabdina*; its surface bears fine, close-set, inconspicuous striations; just behind the epimerite, lines running transversely to these may sometimes be seen.

The nucleus lies about the centre of the body; its diameter is 22.5μ in a large specimen. It is crammed with minute chromatin granules, arranged in strings or scattered more irregularly; often the granules form three or four small clumps. There is usually one fairly large karyosome, which is often pale, but sometimes stains more darkly; in addition to this there may be a smaller karyosome.

The anterior end of the main segment narrows to a small neck, whence arises the epimerite. The surface of this transparent structure stains rather deeply with iron haematoxylin. As we have said, it is always deeply embedded in a cell, a condition of things very unlike what we have noted in the previous species. As the gametocytic stage approaches, the epimerite is easily lost, and isolated epimerites lying within shrivelled epithelial cells may often be seen in sections of the gut. The epimerite (fig. 16, Pl. 20) measures about one-sixth of the total length of the full-grown gregarine; it is a broadly spreading umbrella-like structure, of which the margin is divided into four or five lobes, each fluted on the anterior surface and elaborately frilled around its edge. The fluting of the lobes is continued on to the rounded apex of the umbrella as a series

of converging striations. It is sometimes possible to see that a slight pellicular ridge stands up from the posterior segment around the base of the neck of the epimerite. Just anterior to the epimerite in the fixed and stained host cell, there usually lies a vacuole crossed by a few protoplasmic strands. *

Individuals about to associate shorten and thicken. As we have said, they generally lose their epimerites before associating, but occasionally they may associate precociously (fig. 22, Pl. 21). Association is lateral, and the pair are usually disposed head-to-tail. Very commonly they are of different sizes. When attachment has been effected by circular areas on the apposed sides, the gametocytes become almost spherical, so that their combined bodies have the outline of a figure 8 (fig. 23, Pl. 21). At this stage their pellicular striations fade, and it becomes impossible to distinguish the associated pairs of *Hentschelia* from those of its fellow gregarines, except by the nuclear structure. In this condition they are passed out into the sea-water with the faeces of the worm.

B. *Lecythion thalassemae* n.gen., n.sp.

Attached to epithelial cells in the same part of the gut with *Hentschelia* we find, though less frequently, another dicystid. This organism, when full-grown, has the form of an elongated soda-water bottle, of which the epimerite represents the neck and cork¹ (fig. 17a, Pl. 20).

Adult trophozoites measure about $135\mu \times 52\mu$; the epimerite contributes about one-fifth of the length, or sometimes less. The posterior segment is often pointed posteriorly, so that its outline is that of a lemon. The epimerite (fig. 21, Pl. 20) is a flexible, thin-walled structure; in half-grown specimens it is longer in proportion to the posterior segment than it is in the older trophozoites; its greatest width is about 11μ . After leaving the main segment it swells out slightly, then narrows again, and finally expands at its tip into a slight cone, the cork of the bottle, around the base of which are some fourteen or fifteen

¹ It has considerable resemblance to the tricystid gregarine *Phialoides* Léger.

petal-shaped lobes, which, tilted on edge, grip the epithelial cells and serve as an anchor for the gregarine. The surface of the epimerite is longitudinally ridged; these striations thicken and become much more obvious at the proximal end, where they curve down on to the deutomerite, following there the line of a right-handed spiral. The 'cork' bears a number of minute denticles; and similar, but larger, denticles project from the obliquely running lines on the pellicle of the deutomerite; the tips of these deutomeritic teeth are directed backwards (fig. 17b, Pl. 20). The cytoplasm within the epimerite is hyaline, but in the terminal cone region are a few minute granules, and a group of larger, highly refringent granules, two to eight in number, lies near its posterior end. These granules stain deeply with iron haematoxylin.

When the gut is teased for the making of smears, the trophozoites tend to come away entire, leaving behind them the cells to which they have been hanging. This is in marked contrast to what happens with *Hentschelia*. The explanation is that the epimerite of *Lecythion* merely clamps its gregarine to the cell-surface, as do the claws on the epimerite of *Polyrhabdina*; in the adult condition, anyhow, it is never intracellular. The host-cell is scarcely, if at all, hypertrophied. The point of attachment of the epimerite is generally at the bottom of a fold in the intestinal wall, and the epimerite is supported on two sides at least by the surrounding epithelium, only the deutomerite hanging out quite freely into the lumen. When alive, and while still attached to the gut-wall, the trophozoites show certain 'metabolic' movements which oddly resemble those of *Thalassema* itself. A constriction appears behind the epimerite (as it does just behind the proboscis of the echiurid) and gradually travels backwards to the posterior end; as it fades away, another wave of contraction begins at the anterior end; the direction of these peristaltic waves is never reversed. When detached from its hold, the gregarine is also capable of movement from place to place. It glides slowly along, usually hind-end foremost. This movement is not accompanied by any contractions of the body-wall that we can detect, and it is difficult to see how the backwardly directed

denticles on the pellicle can assist progression by gripping the slimy substratum.

Though we have examined many sections of the gut of infected *Thalassema*, we have never found in these any evidence that *Lecythion* has an intracellular stage. In one smear, however, from a heavily infected worm, we saw what is shown in fig. 20 (Pl. 20). Here in a piece of detached epithelium two young gregarines are embedded, and they appear to be lying in vacuoles within the tissue, though whether they are between cells or actually intracellular it is impossible to say with certainty. The epimerite, still very short, seems to be telescoped into the main segment; within it the characteristic siderophilous granules show clearly, and the lobes around the 'cork' are just appearing. These young parasites measure $12-27\mu \times 9-21\mu$. All later stages that we have seen are definitely extracellular and lie in the gut lumen. The epimerite seems to grow very quickly at first, and in a specimen 100μ long measures about one-quarter of the total length. The little teeth on the pellicle are relatively much larger at this stage, and we conclude that they do not increase in size as the deutomerite grows.

When full-grown, the gregarines lose their hold on the epithelium and associate in pairs. Usually the members of a pair are of approximately the same size. They lie side by side and head to tail (fig. 31, Pl. 21). The epimerite persists for a time; it gradually curves over on to the free side of the main segment (fig. 32, Pl. 21), and is apparently absorbed; we have never found that it is cast off entire as in the last species. The gametocytes become firmly attached to one another by circular areas in the middle of their apposed sides; they then shorten and thicken, so that the pair has the outline of a figure 8. For a time it is possible to detect the characteristic denticles on the pellicle; but when these disappear, we cannot distinguish the association stage of this gregarine from that of its fellow dicystid, except by the nuclear structure in stained preparations; for a time after association the gametocytic nuclei of *Lecythion* stain much more faintly and evenly than those of *Hentschelia* and still show the two karyosomes, one much smaller and more siderophilous than the other.

In this condition the paired gametocytes are voided with the faecal balls, and their further development must take place in the sea.

SPORE FORMATION IN GREGARINES FROM THE GUT OF THALASSEMA.

We hoped to be able to trace the spore formation of our gregarines. But though we have found gametocysts showing nuclear division, though we have seen gametes and watched them copulate, and though we have found ripe sporocysts and counted the sporozoites they contain, we are unable to state with certainty to which species of gregarine these various stages belong.

When they leave the body of the host, the paired gametocytes of *Hentschelina* and of *Lecythion* are, as we have said, indistinguishable from one another except when stained, and then only if the nuclei are unchanged. And the problem is complicated by the fact that a peculiar acephaline gregarine which may also be present in the gut of *Thalassema* has dimensions comparable with the gametocytes of the two dicystids there. We have only twice seen this third gregarine associating, and then in sections of a worm that contained no gregarines but the acephaline, whereas the dicystids seem to associate very readily. Accordingly, we might infer that most of the gametocysts we find mixed up with the excrement belong to one or other of the dicystids; but we are bound to admit that this is mere conjecture: the acephaline may have secret bouts of association that we have not detected. For it is not possible to observe through the thick body-wall of a living worm what are its intestinal parasites; and as we have had to rely for our study of spore formation on what we found casually in the deposited faecal balls, we may have been dealing with an assemblage of three types of gametocyst belonging to as many different species. Investigation is further rendered difficult by the fact that, as the specimens of parasitized *Thalassema neptuni* almost always come from relatively deep water, their gregarines must be accustomed to forming their spores in conditions that we cannot reproduce satisfactorily in the laboratory.

Indeed, for a long time we could not trace further development in any of the ejected pairs; their nuclei sometimes elongated and became longitudinally folded on the surface; but after that they lay unchanged in the sea-water for some days and then disintegrated. But we continued our search, and we have now a number of isolated facts to record, though whether these should be pieced together to make one developmental story is quite uncertain. It seems important, we notice, to examine the faecal material as soon as possible after the worms have come to the laboratory; gametocysts found then seem to be more healthy and show greater power of further development when put in a moist chamber for observation than those collected later on. Moreover, the echiurids rather quickly become defaunated and the chances of obtaining numerous associating pairs becomes less and less after a few days.

After they have been in the water for some hours, the paired gametocytes, forming together a figure of 8, become more closely adpressed along the line of contact, and, where they touch one another, their surfaces tend to become interfolded. The 'waist' of the figure 8 thus disappears and the combined bodies have an elliptical or even a circular outline. A membrane appears surrounding this, and beyond the membrane is secreted a relatively thick, transparent envelope (fig. 24, Pl. 21), which seems to be gelatinous and sticky, for bacteria and other débris tend to adhere to its surface. A comparable envelope is formed round the gametocytes of *Nina* (*Pterocephalus*) (see Léger and Duboscq (1909)). On fixation this outer envelope shrinks very much (figs. 26 and 28, Pl. 21).

Sections of fixed and stained cysts in a late figure 8 stage show that the nuclei of the gametocytes become broad spindles, with the chromatin arranged in strings of granules stretching from pole to pole; the nuclear membrane is much folded and we get the impression that fluid has passed from the nucleus into the cytoplasm. We have not seen the completion of the first nuclear division. The next stage we find shows the surface of the gametocytes becoming hummocky, and it is possible to detect, even in the living condition, that a fairly large number of nuclei are disposed near the periphery. Fig. 26 (Pl. 21) is drawn

from a whole mount of such a cyst; it can be seen that some of the nuclei are dividing mitotically. Fig. 27, *a*, *b*, *c*, *d* (Pl. 21) shows some of these mitotic figures, drawn from sections of an older gametocyst; in a number of these spindles it appears that there are only three chromosomes.

The gametes are very slow to develop; but, as we have said, the laboratory conditions must be far from what is normal. The gametes appear as clear beads on the surface (fig. 24, Pl. 21), each containing a few refringent granules; in the centre of the group there is a large, dense, residual mass. Each gamete measures $4.5\text{--}6\mu$ in diameter; those from one gametocyte are slightly larger than those from the other. In one specimen kept under observation in the moist chamber, spherical gametes were noticed at 11.30 a.m., and at 4 p.m. that day they were in pairs; the copulae, however, developed no further. In another cyst in which the gametes had just formed, we noticed peculiar movements; and while we were observing it the envelope ruptured and the gametes escaped. We then saw that the two kinds differed from one another not only in size, but in structure and behaviour. The smaller ones, measuring about 4.5μ in diameter, remained inert; whereas the larger ones, about $5\text{--}6\mu$ in greatest diameter (fig. 29 *a*, Pl. 21), had become pear-shaped and moved about by means of a fairly stout flagellum arising from the more pointed end. This flagellum was twice, or even three times, the length of the body, and, as the gamete moved, the flagellum was carried in advance. The body itself also showed 'metabolic' movements, and the whole thing curiously resembled the flagellated stage of certain mycetozoa. The flagellum was sometimes absorbed, and then the gamete continued to move actively from side to side the clearer, pointed prominence whence the flagellum had vanished. We saw some of the flagellated gametes attach themselves to the smaller, inert gametes and gradually fuse with these (fig. 29 *c*, Pl. 21); the copula continued to move for some time by means of the gradually shortening flagellum that projected from it.'

The sporocysts found in another cyst were approximately spherical and measured $9\text{--}10\mu$ in diameter. The envelope was quite well developed, but without any trace of polar thickenings.

There was a small residual group of granules in the interior, and we could see the sporozoites around it, but they lay so twisted within the sporocyst that we could not count them with certainty. We treated some of these ripe sporocysts with Weigert's iodine solution, and one of them burst, the contained sporozoites spreading out then in such a way that it was easy to see that there were eight (Pl. 29 e).

We have had so small a material to deal with that we can give no detailed information about the gametic structure.* We stained smears of the copulating gametes described above, and we cut sections of two other cysts that contained gametes; but the results were not very helpful. We expected to find that the male, flagellated gametes would conform to the plan of structure revealed by Léger (1904) and by Léger and Duboscq (1909) in some tricystid gregarines in which there is pronounced anisogamy; and it may be that, with better stained preparations, we should get results more closely comparable with those of the French protozoologists. Admittedly, the copulating gametes we observed were in conditions somewhat liable to produce abnormality of form, for sea-water must have got mixed with the contents of the ruptured gametocyst, and we cannot believe that rupture of the cyst before sporocyst formation is at all usual. But they moved about actively and found their partners as though they were quite healthy. Accordingly we were puzzled to notice that the male gamete moves flagellum-end first, whereas it would appear that in Léger and Duboscq's gregarines, the flagellum is posterior. We found a gametocyst with developing gametes, in which one of the gametocytes was covered with fine, hair-like, cytoplasmic processes (fig. 25, Pl. 21). These were immobile and looked like the outgrowths on the surface of certain neosporidians. If they were, as we should be inclined to suppose, the sprouting rudiments of the flagella, then the flagellar end of the male gametes here is certainly the reverse of what it is in *Stylorhynchus* and other anisogamous gregarines that have been observed; for in these the flagellum develops from the drawn-out region of attachment of the male gamete to the residual mass behind it.

THE SYSTEMATIC POSITION OF THE DICYSTID GREGARINES.

At one time there was a tendency among systematists to treat the dicystid gregarines as a group very distinct from other cephalines. Brasil (1909) may have been right in supposing that *Doliocystis* is more nearly allied to the acephalines than it is to true septate forms, and Kamm (1922), followed by Bhatia (1980), definitely placed *Lecudina* (*Doliocystis*) among the *Acephalina*. On the other hand, the epimerites of *Schneideria*, *Sycia*, *Polyrhabdina*, *Hentschelia*, and *Lecythion* are as complex as those of any tricystid.

Léger (1892) with his usual good sense remarked, 'Il résulte de ces observations que les différentes formes de dicystidées vraies se relient aux polycystidées par des transitions insensibles, et que la présence d'un septum ne peut constituer un caractère morphologique sérieux, susceptible d'être invoqué comme base d'une classification des Grégarines.' We think that there is justification for Léger's opinion and that it needs fresh emphasis.

Kamm (1922), as we have said, did not regard *Lecudina* (*Doliocystis*) as a true dicystid. She created a new polycystid family, *Polyrhabdinidae*, to include *Polyrhabdina*, *Sycia*, and *Ulivina*. The definition runs as follows: 'Polycystid (septate) gregarines inhabiting the digestive tract of polychaetes. Epimerites varied,' and she adds the somewhat inconsequent remark that they 'stand near the border-line with the *Acephalina* because of their presence only in polychaetes'. *Schneideria*, being a parasite in insects, finds no place here. The new genera we have described might fall within the definition of *Polyrhabdinidae* given by Kamm, since the echinurid worms are generally regarded as allied to the polychaetes. But our observations show that *Hentschelia*, at any rate, differs very much in its behaviour from *Polyrhabdina* and from *Sycia*.

It is abundantly clear that the dicystid gregarines are a heterogeneous group. Some occur in insects, others in polychaete worms, and we now record two from an echinurid. Some, such as *Doliocystis* (*Lecudina*?) and *Hentschelia*, are

intracellular in the early stages of their life within the gut, and their epimeritic segment always remains intracellular; the indications are that in others, such as *Polyrhabdina* and *Lecythion*, the intracellular stage, if it occurs at all, is transitory, and the epimerite of the adult is certainly extracellular. *Doliocystis* tends to lose its epimerite early; *Lecythion* retains the epimerite even at association. *Doliocystis*, *Sycia*, and *Schneideria* apparently form their spores while still in the host's body; whereas the dicystids from *Thalassema*, and probably also the species of *Polyrhabdina* in *Scolecopsis* and *Polydora*, require the stimulus of sea-water before they can proceed far with sporogony.

Parallel differences exist between tricystid gregarines. In some tricystids the epimerite lies within the host-cell; in others, such as *Nina* and its allies, the epimerite is external to the cell. Genera differ from one another in the degree of development and in the persistence of the epimerite. The majority form their spores within the host's body; but in some, such as *Phialoides*, the cysts do not mature until they are in the water in which the infected animal lives.

Until protozoologists will be at the trouble to examine for themselves the gregarines so inadequately described by Mingazzini and his contemporaries, they cannot be in a position to deal with the question of classification. The confusion that has arisen over the nomenclature, for instance, is mainly due to later authors having quoted uncritically statements made at a time when gregarines were scarcely known. Only a thorough knowledge of the life-histories will enable us to judge of the affinities of the dicystid gregarines with one another and with other forms. The fact that, among dicystids, *Sycia* and *Polyrhabdina* are infected with species of *Metschnikovella* whereas *Hentschelia* and *Lecythion* are never parasitized in this way, may have its significance; but in determining possible relationships of such gregarines it is obvious that knowledge of the spore structure is the matter of prime importance.

SUMMARY.

1. Two species of the dicystid gregarine *Polyrhabdina Mingazzini* have been studied in polychaete worms from Plymouth.

(a) *Polyrhabdina spionis* is not the organism described by K  lliker as *Gregarina spionis*, and should be named *Polyrhabdina spionis Mingazzini*.

(b) We do not consider that the epimerite of *Polyrhabdina spionis* is intracellular, nor do we believe that it is an amoeboid and food-absorbing structure. The specimens we have found in *Scolecopsis fuliginosa* differ in other details from those described by Caullery and Mesnil in the same worm from another locality, and may constitute a new variety.

(c) The dicystid gregarine from the intestine of *Polydora flava* is described by us. This may be the organism called by Caullery and Mesnil *Polyrhabdina polydorae*, but we do not agree that this name is synonymous with *Doliocystis polydorae* L  ger.

2. We describe two new dicystid gregarines from the intestines of the echiurid worm *Thalassema neptuni* G  rtner at Plymouth. These we have named *Hentschelia thalassemae* n.gen., n.sp., and *Lecythion thalassemae* n.gen., n.sp.

3. Association was frequently observed in the gregarines from *Thalassema*. The gametocytes do not normally develop further until they have been passed out with the excrement. In sea-water a cyst is formed and spores developed. Anisogamy, with flagellated male gametes, has been observed.

4. The dicystid gregarines are a heterogeneous group. Those with simple, transitory epimerite may be related to the acephalines; but the species dealt with in this paper are almost as complicated in structure as tricystids, and seem to differ from these only in the absence of a septum dividing a protomeritic from a deutomeritic segment.

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EXPLANATION OF PLATES 20 AND 21.

All figures drawn either from living specimens or from smears and sections fixed in Brasil's modification of Bouin-Duboscq's fluid and stained with Heidenhain's iron haematoxylin. A camera lucida was employed. The microscope was a large Leitz model, fitted with 1/6" and 1/12" (oil immersion) objectives and oculars 4, 8, and 12 (these last two compensating oculars of Zeiss).

PLATE 20.

Polyrhabdina spionis n.var. *bifurcata*.

- Fig. 1.—Trophozoite. Drawn from a smear. $\times 350$.
- Fig. 2.—Young trophozoite. Drawn from a section. $\times 350$.
- Fig. 3.—Transverse section of a trophozoite. $\times 1,000$.
- Fig. 4.—Epimerite of the gregarine attached to the epithelium (cilia omitted). The nearer wall has been shaved away in the section. $\times 1,400$.
- Fig. 5.—A more slender trophozoite parasitized by *Metchnikovella spionis*. The parasite is in the hyphal stage. $\times 350$.
- Fig. 6.—A trophozoite crammed with the cysts of *Metchnikovella*. The spores are shown in two cysts only. $\times 350$.
- Fig. 7.—Cyst of *Metchnikovella spionis* from *Scolecopsis fuliginosa*. $\times 1,400$.

Polyrhabdina polydora (?).

- Fig. 8.—Large trophozoite attached to the epithelium (cilia of the cells omitted). Drawn from a section. $\times 350$.
- Fig. 9.—Epimerite, showing detail of structure. $\times 1,400$.
- Fig. 10.—Transverse section of a trophozoite filled with the cysts of *Metchnikovella caulleryi*. Note the degenerate nucleus of the host. $\times 350$.
- Fig. 11.—Portion of the endoplasm of a parasitized trophozoite showing

the hyphal stage of *Metchnikovella caulleryi*. From a section. $\times 350$.

Fig. 12.—Later stage in the hyphal development of the parasite; the hypha is becoming segmented into bead-like spores. From a section. $\times 1,400$.

Fig. 13.—Cyst and spores of *Metchnikovella caulleryi*: (a) the entire cyst, $\times 667$; (b) one end of the cyst, $\times 2,000$; (c) three spores, $\times 1,300$.

Hentschelia thalassemae n.gen., n.sp.

Fig. 14.—Young intracellular trophozoite. From a section. Note the hypertrophy of the host-cell and its projection into the lumen of the gut. $\times 1,000$.

Fig. 15.—Mature trophozoite. From a smear. Note the intracellular epimerite. $\times 350$.

Fig. 16.—End-on view of the epimerite. $\times 933$.

Lecythion thalassemae n.gen., n.sp.

Fig. 17a.—Mature trophozoite, attached to the epithelium. From a section. $\times 400$.

Fig. 17b.—A small portion of the pellicle of the same, showing the characteristic spines. $\times 1,400$.

Fig. 18.—Transverse section of a trophozoite in the region of the nucleus. $\times 600$.

Fig. 19.—Young trophozoite, with epimerite still very short. From a smear. $\times 550$.

Fig. 20.—Two young intracellular (?) gregarines. From a smear. $\times 667$.

Fig. 21.—Epimerite of a full-grown gregarine, greatly enlarged to show detail of structure. Note the method of attachment to the epithelial cell (cilia omitted). $\times 1,400$.

PLATE 21.

Fig. 22.—*Hentschelia thalassemae*. Associating gregarines. Note that one still retains its intracellular epimerite. The nuclei are elongating. From a smear (striations omitted). $\times 500$.

Fig. 23.—*Hentschelia thalassemae*. Association; figure 8 stage. From a smear. $\times 500$.

Fig. 24. Living gametocyst of (?) some hours after evacuation from the worm. Note the thick ectocyst. Gametes are developing on the surface of the gametocytes, and those from one are rather larger than those from the other. $\times 500$.

Fig. 25.—Another living gametocyst, in which one of the partners is covered with fine, hair-like processes. $\times 500$.

Fig. 26.—Fixed and stained gametocyst, showing nuclei in process of division. From a smear of the evacuated faecal balls of *Thalassema*. $\times 500$.

Fig. 27, a, b, c, and d. Four stages in the division of nuclei in an older

gametocyst. From a section. In two of the figures it can plainly be seen that the number of chromosomes at this stage is three. $\times 2,100$.

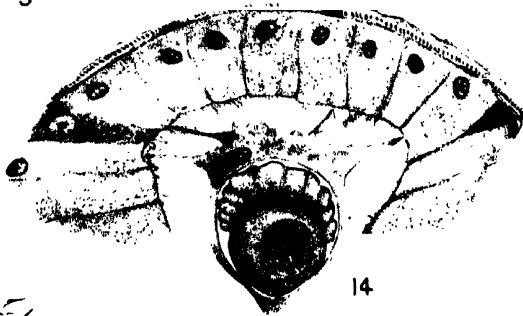
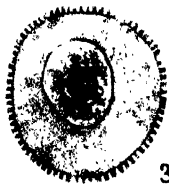
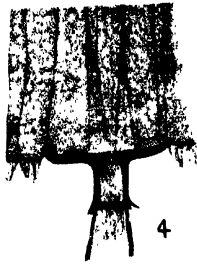
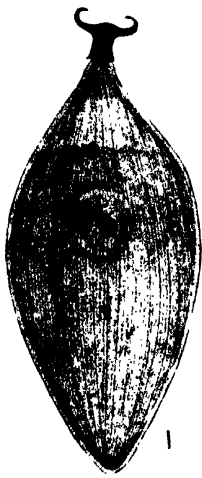
Fig. 28.—Section of a gametocyst in which gamete-formation is finished. The gametes are fusing in pairs, and some of these show a flagellum. A few sporoblasts are shown, and one finished spore. In the centre of the cyst lies the residual body. (Only a few of the gametes that appeared in the section have been drawn.) $\times 700$.

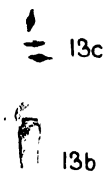
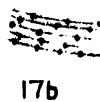
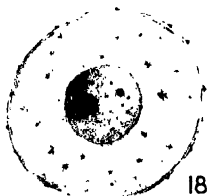
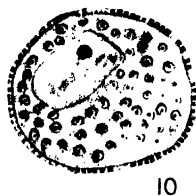
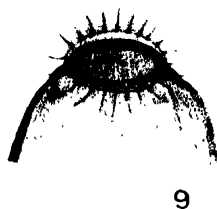
Fig. 29.—Living gametes: (a) flagellated male gametes; (b) passive female gamete; (c) and (d) copulae; (e) ruptured sporocyst showing the eight sporozoites. $\times 1,800$.

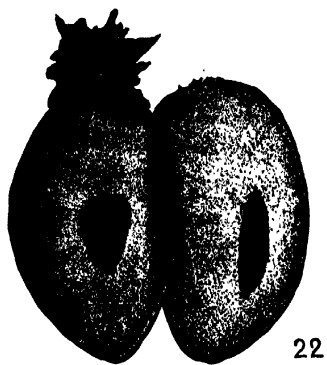
Fig. 30.—Sporoblast (fixed and stained) containing eight nuclei. $\times 1,600$.

Fig. 31.—*Lecythion thalassemae*. Association. From a smear. (Pellicular striations omitted.) $\times 500$.

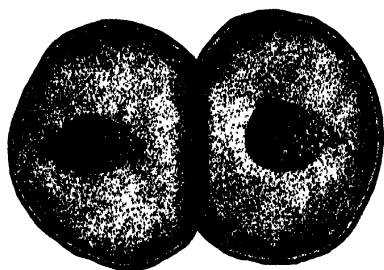
Fig. 32.—A slightly later stage, in which the epimerites have folded over and are being absorbed. From a smear. (Striations omitted.) $\times 500$.



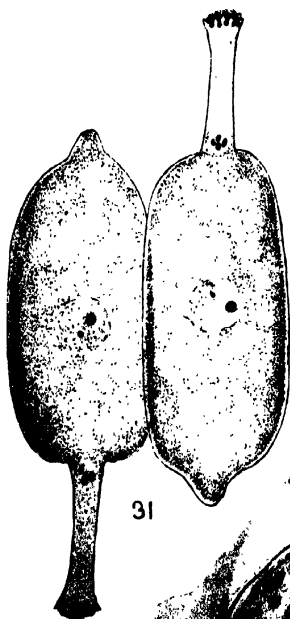




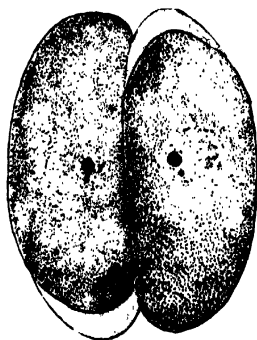
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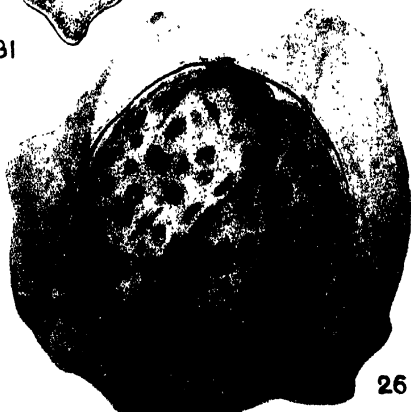
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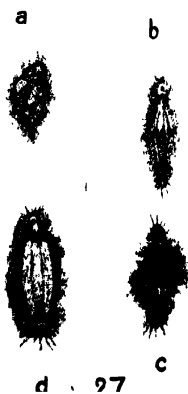
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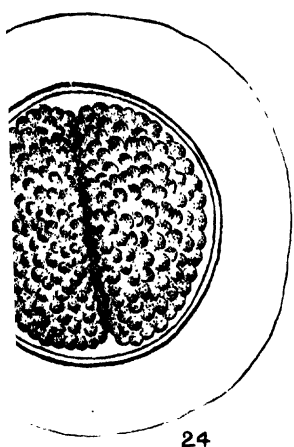
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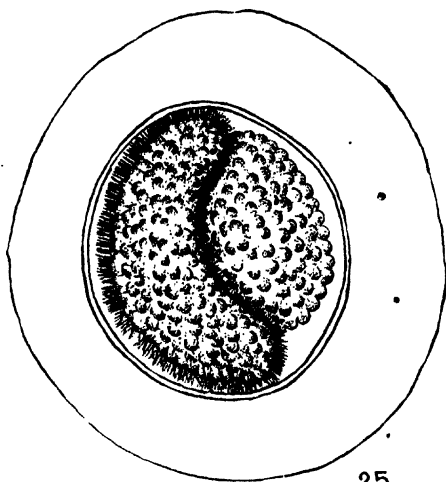
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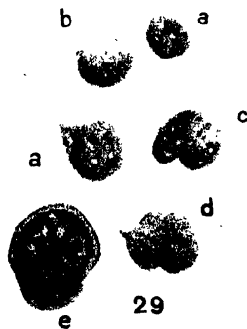
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**A New Protozoon, *Hyperidion thalassemae*
n.gen., n.sp., from the intestine of *Thalassema neptuni* Gärtner.**

By

D. L. Mackinnon, D.Sc.,

and

H. N. Ray, Ph.D.

From the Zoological Department, King's College, London.

With Plate 22.

IN the spring of 1930 we found in the intestine of the echiurid worm *Thalassema neptuni* at Plymouth a peculiar protozoan organism which seems worthy of some description. During the summer term we had additional specimens of the worm sent to us from Plymouth and kept them under observation in the laboratory, but we learnt little more about the parasite than we had already gathered from our earlier observations. We examined, in all, 145 worms: 20 per cent. of those dredged in the sound were infected, but we never found the parasite in those taken between tide-marks.

The parasite is attached to the cells lining that portion of the gut which is accompanied by the 'collateral intestine', and it is especially abundant in the thicker-walled region thereof. Along with it there often occur two dicystid gregarines which we have described elsewhere (Mackinnon and Ray, 1931); the holotrichous ciliate *Ptyssostoma thalassemae* is also a common parasite.¹

So far, we have seen only the young and what we suppose to be the adult stages of the trophozoite; and until the fertiliza-

¹ Lankester's 'gregarine' from *Thalassema neptuni*, first noted in 1881, infests the eggs in the genital pouches. It is really a coccidian (Mackinnon and Ray, 1929).

tion is known and the method of spore formation, it is of course impossible to classify the organism accurately. But appearances strongly suggest that it is a peculiar acephaline gregarine, and the two gametocysts we have seen that may belong to the sexual part of its life-history seem further evidence for that view.

METHODS.

The living worms look quite healthy and it is impossible to judge from their outward appearance whether they harbour the parasite. We dissected out the gut quickly and examined it at once; under the slight pressure of a cover-glass, we could easily see the parasites through the walls of the intestine, and a little practice enabled us to distinguish the acephaline gregarine from the accompanying dicystids, which are of much the same size, and one of which, *Hentschelia thalassemae*, is attached to the gut wall in a similar way. So far as was possible, we studied the gregarine while it was in these undisturbed conditions; then we opened a portion of the gut and observed the trophozoites that fell out. Smears were made of the epithelium and of the gut-contents, and these were fixed and stained. If the infection were pretty heavy, we then fixed selected portions of the intestine and cut sections, 6–8 μ thick.

Schaudinn's fluid was used as fixative sometimes; but we got the best results with Brasil's modification of Bouin-Duboscq's fluid. Smears and sections fixed in these ways were stained with Delafield's haematoxylin (sometimes followed by borax-carmin) or by Heidenhain's iron haematoxylin followed by various counterstains, of which chromotrop 2R proved the most convenient. Dobell's modification of Mann's methyl blue eosin was also used with success. Some of the material was fixed with Helly's fluid, and then stained in Altmann's acid fuchsin and toluidin blue.

DESCRIPTION OF THE PARASITE.

The youngest stages were seen only in sections of the gut. The parasite then appears as a sub-spherical, intracellular body, measuring about 10–21 μ in diameter, and surrounded by a vacuole (fig. 1, Pl. 22). Its nucleus, which is relatively enormous

at this stage, contains one or two large karyosomes. On one side of the body, and generally towards the lumen of the gut, we could sometimes see a sausage-like projection folded against the main mass; occasionally this outgrowth was transversely ridged. These young stages are very scarce in our preparations, and we have not seen the condition intermediate between this and the, presumably, adult parasite. This appears as a pestle-shaped body, of which the thickish 'handle' projects into the lumen, while the main mass (the 'head' of the pestle) lies buried in a greatly enlarged epithelial cell; if the parasite be likened to a pestle, then the host-cell is the mortar (figs. 2 and 3, Pl. 22).

The parasitized cell presents at this stage a very extraordinary appearance. Not only does it become hypertrophied, but it rises up beyond its fellows until it swings clear of the general level of the epithelium and hangs into the gut, though it remains tethered to the wall by four or five rooting protoplasmic threads. As the outgrowth of the gregarine proceeds, the cell becomes reduced to a mere skin around it, but the rooting processes from its base persist as effective anchors, and its nucleus, though somewhat hypertrophied and flattened, is always recognizable.

We shall speak of the implanted, rounded end of the gregarine as its anterior end, from analogy with what is the affixing end in cephaline gregarines; though whether we are justified in this interpretation can be decided only after observation of the sporozoite and its mode of entry into the epithelium. At its point of emergence the body of the parasite shows a marked constriction, and around this the edge of the 'mortar' stands up like a collar. The portion of the gregarine that is exposed might be described as a sort of proboscis, were it not that this is probably the hind end; beyond the constriction at its base, this projection swells out considerably and then tapers somewhat abruptly to a point.

The endoplasm is granular, and appears opaque and greyish when seen by transmitted light in the living condition. It stains yellowish-brown with iodine solutions. The nucleus may lie either in the intracellular portion or in the 'handle', or mid-way between these; we get the impression that it may be squeezed backwards and forwards with the body's contractions, and it is often

deformed from the spherical (fig. 2, Pl. 22). It contains many chromatin granules suspended in the nuclear sap, and at this stage there is usually one karyosome, around which the chromatin granules are often more densely congregated. The karyosome stains black with iron haematoxylin and red in Mann's stain. There is a well-defined nuclear membrane.

The cytoplasm forms a distinct pellicle, which is thickest just below where the handle joins the head of the pestle. In the lower layers of the ectoplasm, but in the handle region only, run longitudinally disposed, dark-staining lines beneath the pellicle, which seem to be contractile elements (fig. 4, Pl. 22). The pellicle on the implanted region may be slightly furrowed.

This is the appearance presented by the fully expanded, full-grown organism. Trophozoites in this condition measure from 53μ to 145μ in length; of this measurement the exposed portion accounts for rather more than half, as a rule. The width of the implanted portion of such specimens is 22.5 – 60μ ; the width of the handle varies from 11 – 53μ , depending on the degree of its extension. The nucleus is about 15 – 23μ in diameter in large individuals, and is relatively much larger in small ones.

The projecting handle region is contractile. The movement is apparently very slow; we have watched extended gregarines for long periods and they have only very slightly shortened and thickened during that time. The addition of small quantities of acid to the gut contents seems to kill the organism before it has contracted more than a short way. But in sections of the gut it is common to find gregarines with the handle partially contracted, and a large number in which it has withdrawn altogether. As the myonemes beneath the pellicle shorten, that layer is thrown into circular folds, five to ten in number and varying very much in width; distally the tip of the handle protrudes unwrinkled (figs. 5 and 6, Pl. 22). The contraction continues, and the thicker pellicle at the proximal end of the handle tends to stand up around it as a ridge. Finally, the whole 'proboscis' is flattened on to the spherical, intracellular portion, which by this time has, of course, increased considerably in volume. A completely contracted gregarine is practically withdrawn into the host-cell; on its free side appears what looks like an elabor-

ately lipped sucker, which almost blocks the entrance (fig. 7, Pl. 22). This sucker is the retracted, flattened-out handle portion. Seen end-on, it shows a clear area in the centre, which marks the tip of the handle; around this are several concentric circles, radially striate, which represent the flattened-down, pellicular folds; and beyond these again is the rim of stiffer, thicker pellicle at the handle base (fig. 8, Pl. 22). This rim tends to be lobed and the margins of the lobes may show flutings.

We should judge that, except in its very young stages, the parasite does not depend for nourishment on the host-cell, which serves almost solely as a means of anchorage to the gut wall. The exposed region is probably responsible for absorbing fluid food from the gut contents.

Retracted, spherical gregarines are always much more common in the posterior part of the intestine, and possibly they are preparing for association. When in the contracting stage they have a strong resemblance to the figures given by Léger and Duboscq (1909) of the cephaline gregarine *Nina* (*Pterocephalus*) just prior to pairing. Pairs of associated gregarines are very common in the posterior part of the intestine of *Thalassema*; but, so far as we can judge, these are the gametocytes of the accompanying dicystids, *Hentschelia* and *Lecythion* (Mackinnon and Ray, 1931). Where one is dealing with three different gregarines of similar dimensions, all living together and apparently not proceeding to spore formation until liberated from the host, it is obviously a matter of very great difficulty to distinguish between them when the sexual phase comes on, for then they tend to lose such morphological characters as enable one to tell them apart readily enough when they are trophozoites. In sections of one *Thalassema*, heavily infected with the acephaline and, so far as we could discover, containing neither of the other gregarines, we did find free in the lumen of the gut two groups of paired gametocytes, measuring $145\mu \times 70\mu$, which we think may be referable to *Hyperidion* (fig. 9, Pl. 22). The gametocytes were of equal size and each showed fine pellicular striations which converged towards its free end, where was a slight depression surrounded by a rim. If this crater at the free end

of the gametocyte is the last remnant of the contracted handle of the pestle, then, according to our interpretation, these gregarines associate by their anterior ends, whereas the accompanying dicystids associate laterally. The nucleus in each gametocyte was drawn out into a broad spindle shape and filled with chromatin granules arranged in longitudinally disposed lines. We know nothing about the further development, and are unable to say whether the peculiar flagellated male gametes observed in certain gametocysts passed by *Thalassema* (Mackinnon and Ray, 1931) belong to the life-cycle of this gregarine; but we think it unlikely.

SYSTEMATIC POSITION.

True acephaline gregarines, other than schizogregarines, are very rare inhabitants of the alimentary tract. Probably the species of *Lankesteria* are the only gut 'monocystids' of which the life-cycle is known in any detail. *Doliocystis* (*Lecudina*) is by some authors classed as a 'monocystid', by others as a 'dicystid'; in any case, it has a fairly well-developed epimerite, and shows no resemblance to the parasite from *Thalassema*. Hesse (1909) mentions *Monocystis mitis* Leidy from the intestine of the oligochaete worm *Fridericia polycheta*, but his account and the pictures he gives incline us to think that this organism may really be related to *Selenidium*. Earlier observers, such as Diesing (1850) and Frenzel (1885) recorded monocystid-like gregarines from the gut of Crustacea; but it has since been shown that these were cephaline forms detached from their epimerites. It is very difficult to know what should be the systematic position of the monocystid genera described by Mingazzini (1891 and 1893) from the alimentary tract of various marine animals from the Gulf of Naples. We have read the descriptions and looked at the figures he gives, but there is nothing that agrees with what we have found in *Thalassema*. In fact, the only gregarine that bears even the slightest resemblance to our parasite is that described by Greeff (1880) from the gut of *Echiurus pallasii*, and named by him *Conorhynchus gibbosus*. Labbé (1899)

pointed out that the name *Conorhynchus* was preoccupied, and renamed Greeff's gregarine *Zygosoma gibbosum*.

Greeff found the parasite of *Echiurus* especially abundant in the spring, and he gives as its most characteristic feature that the adult organisms are almost always associated in pairs, with their posterior ends in apposition; it was doubtless this feature that Labbé had in mind when he renamed the genus *Zygosoma*. There are certain coelomic monocystids that normally occur in pairs in this way, even a long time before spore formation is initiated; and possibly *Zygosoma* may behave like one of these. On the other hand, very little was known about gregarines when Greeff made his observation, and one would like to be sure that he was not merely dealing with an epidemic of association: study of a larger number of worms, and at other seasons of the year, might have shown that the organisms remained single throughout their growth period. We had this in mind, certainly, when we considered Greeff's gregarine from *Echiurus* as perhaps a near relative of the one we find in *Thalassema*. For his description of 'a large proboscis-like outgrowth', somewhat retractile, from the free (according to him, the anterior) end of each gregarine in the pair, suggested some possibility of comparison with what we find in the single individuals of our gregarine. But the proboscis, according to Greeff, may occasionally serve as an organ of attachment. Moreover, the adults are described as completely transparent and filled with 'kleinen wasserhellen Vakuolen'; and the surface of each hemispherical member of the pair is covered with conical and warty processes. The pictures he gives of the organism in this phase are like nothing we have seen in *Thalassema*. The dimensions also—1 mm. in length and as much in breadth—are far in excess of what we have found. A slow, creeping movement is described; our gregarines always remain stationary. There seems to be no cellular implantation in *Zygosoma*. The young stages are said to be like *Monocystis agilis*, but drawn out into a conical process fore and aft, the anterior one usually broader than the one behind; the endoplasm of these young gregarines is opaque and granular, the transparency of the adult condition being only gradually attained.

Greeff's account is so circumstantial and the pictures he gives seem to have been drawn with such care, that we must regard the appearances he describes as typical of the parasite from *Echiurus pallasii*. And as they do not accord with our own observations on the acephaline from *Thalassema neptuni*, we cannot place that in the genus *Zygosoma*, but are obliged to give it a new name. We suggest *Hyperidion thalassemae*, n.gen., n.sp. Reinvestigation of Greeff's gregarine may show that the genera *Zygosoma* and *Hyperidion* are more closely related than appears; but only detailed study of gamete and spore formation will give evidence to decide what should be their systematic position among 'acephaline' gregarines. In the hope that we may eventually light on the spores, we are continuing our investigation; but already we have seen enough of the difficulties to realize that such findings will be a matter of sheer good luck.

We wish to acknowledge our indebtedness to the High Commissioner for India and to the Layton Research Fund of King's College for grants-in-aid awarded to one of us (H. N. R.) during the course of our work.

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EXPLANATION OF PLATE 22.

All drawings were made with the help of a camera lucida from sections of the intestine of *Thalassema neptuni*. Fixation with Brasil's modification of Bouin-Duboseq, and staining with Heidenhain's iron alum haematoxylin, counterstained with chromotop 2 R (except in fig. 9, where the stain was Mann's methyl blue eosin). The microscope employed was a large Leitz model, with objectives 1/6" and 1/12" (oil immersion) and compensating ocular 8 of Zeiss. The magnification is stated in the description of each figure.

Hyperidion thalassemae, n.gen., n.sp.

Fig. 1.—Young intracellular parasite. Note the outgrowth on one side, and the hypertrophy of the host-cell. $\times 2,100$.

Fig. 2.—Half-grown trophozoite. This is a reconstruction from three sections. Note the protruding 'handle' portion. $\times 1,000$.

Fig. 3.—Older trophozoite in longitudinal section. Note the rooting processes of the host-cell, which is reduced to a mere skin round the parasite. $\times 300$.

Fig. 4.—Transverse section of the posterior, retractile portion of a full-grown trophozoite. Note the dark dots which represent transverse sections of the myonemes. $\times 1,000$.

Fig. 5.—Partially retracted 'handle' region. $\times 1,000$.

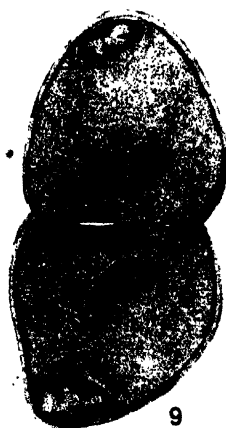
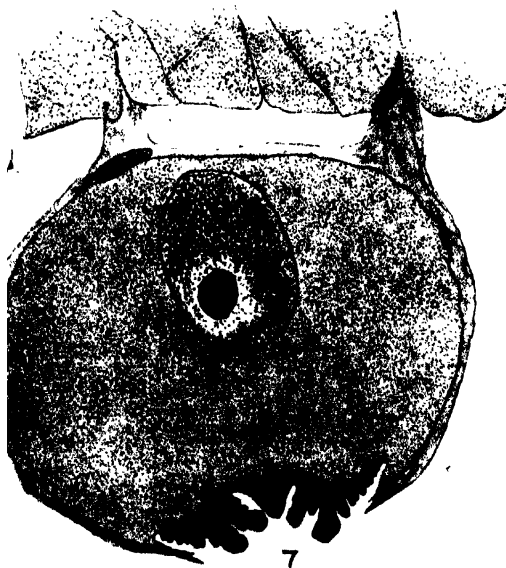
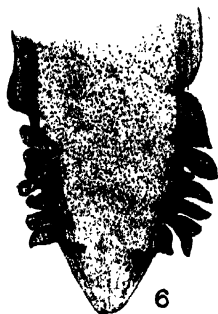
Fig. 6.—The same, in longitudinal section. $\times 1,000$.

Fig. 7.—The organism completely retracted into the host-cell. Note the 'sucker'. $\times 1,000$.

Fig. 8.—End-on view of 'sucker' in a completely retracted specimen. $\times 1,000$.

Fig. 9.—Association in *Hyperidion* (?). Note terminal crater-like depressions, which possibly represent the retracted portions of the gametocytes. $\times 400$.





Mechanisms for the Prevention of Self-Fertilization in some Species of Fresh-Water Triclad.

By

P. Ulyott,

and

R. S. A. Beauchamp.

From the Zoological Laboratory, Cambridge.

With 5 Text-figures and Plate 23.

EXPERIMENTS by Gelei (1924) show that some very effective mechanism for the prevention of self-fertilization does exist in *Dendrocoelum lacteum*. When kept alone from the time of hatching, this species produced cocoons containing eggs which had not been fertilized. Two such animals which had both produced unfertile eggs while isolated, after pairing laid cocoons containing fertile eggs.

The complexity of the genital organs in the fresh-water Triclad's appears to be due to three interrelated causes:

- (1) Hermaphroditism with sperm and ova ripening simultaneously.
- (2) Mutual copulation which provides internal insemination and exchange of sperm.
- (3) The necessity for the prevention of self-fertilization.

The last of these three causes appears to be the most important, and will be dealt with in detail, when it will be seen how far-reaching are the effects it has in modifying the genital organs of different species of the group.

After copulation and insemination further complications arise, due to the formation of a cocoon which contains a number of fertilized eggs and a considerable supply of yolk-cells. The development of the cocoon is not discussed in this paper.

Examination of *Dendrocoelum lacteum* and various

other species, shows that different forms of more or less complex modifications exist which can only find a reasonable interpretation as mechanisms for the prevention of self-fertilization.

The parts of the genitalia of fresh-water Triclad s which show variability are the penis, the muscular gland organ (adenodactyl), the 'uterus', and the genital atrium which is often subdivided. All fresh-water Triclad s possess a 'uterus' in some form or other, but in certain species either the muscular gland organ or the penis may be absent. The form of all these structures must be carefully considered, as must also the point of entry of the oviducts into the atrium, while it is of the utmost importance when deciding how self-fertilization may be prevented to note the exact relative position and size of the different organs in the particular species.

The only reason for employing the term Uterus here is its widespread use in the description of the genitalia of Triclad s. The organ to which the name is applied has functions in no way comparable to those suggested by this name, which was adopted before the purpose of the organ was fully understood; other names by which it has been called are: Drüsenblase, Schalendrüse (Iijima, 1884) (Mattiesen, 1904), receptaculum seminis (Wilhelmi, 1909), Begattungstasche, Gestielter Drüsen-sac (Steinmann, 1913), bursa copulatrix (Burr, 1912).

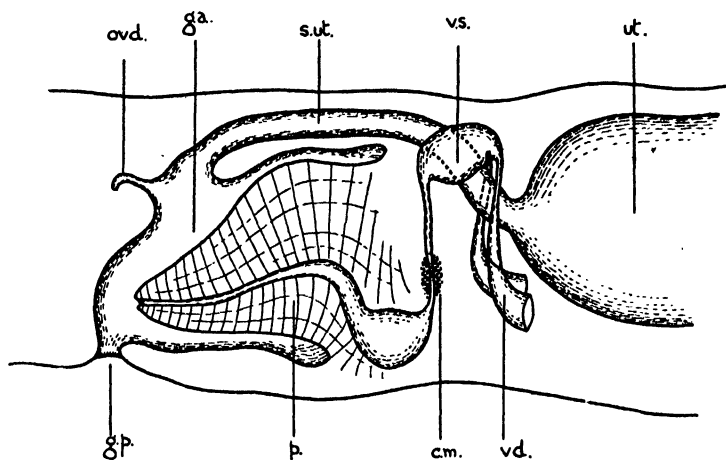
The following description of the genitalia of *Planaria lugubris*, *Dendrocoelum lacteum*, and *Bdellocephala punctata* is based on our own observations. These do not agree entirely with those of previous workers, particularly as regards the arrangement of the organs in *Dendrocoelum lacteum* and *Bdellocephala punctata*.

Planaria lugubris.

In the genus *Planaria* there is typically no muscular gland organ. (The problematic position of *Planaria torva* and allied species cannot be discussed here.) In this genus the penis is very well developed, and the uterus is large with a wide duct capable of accommodating the penis of the co-copulant. In the period of sexual quiescence sperm cannot be found in the penis itself, but is confined to the vasa deferentia and vesicula

seminalis. The restriction of the sperm to these tracts is due to the occlusion of the lumen of the duct leading from the vesicula seminalis by the tonic contraction of the circular muscles, which are present as a layer immediately surrounding the duct itself. In this connexion it is significant that in *Planaria albissima*, which has no vesicula seminalis, the

TEXT-FIG. 1.



Schematic reconstruction of the genitalia of *Planaria lugubris* in a state of sexual quiescence. From series of sagittal and coronal sections. For explanation of lettering see below.

LIST OF ABBREVIATIONS USED IN TEXT-FIGURES AND PLATE.

a.a., anterior atrium; *c.m.*, ring muscles; *d.f.*, dorsal flap; *fl.*, flagellum; *g.a.*, genital atrium; *g.p.*, genital pore; *i.a.*, inner atrium; *i.f.*, iris-like fold; *m.a.*, middle atrium; *m.g.o.*, muscular gland organ; *o.a.*, outer atrium; *ovd.*, oviduct or oviducts; *p.*, penis; *p.a.*, posterior atrium; *p.b.*, bulb of penis; *p.co.*, penis of co-copulant; *p.m.g.o.*, papilla of muscular gland organ; *s.ut.*, stalk of uterus; *ut.*, uterus; *v.d.*, vas deferens or vasa deferentia; *v.s.*, vesicula seminalis; *y.*, duct joining vas deferens to 'penis' (in *Bdellocephala*).

circular layer of muscles referred to is especially well developed (Bohmig, 1909).

The diagram of the genitalia in *Planaria lugubris* (see Text-fig. 1), is reconstructed from serial sections. The penis

is extremely well developed and lies in a curve beginning dorsally, running ventrally, and finally turning posteriorly, so that the distal end lies horizontally and is free in the atrium. Even at rest the penis is large enough almost to fill the cavity of the atrium, which is simple and undivided. The general arrangement of the uterus and oviducts is quite typical for the genus.

One of the functions of the uterus is to receive sperm. This has been disputed by some of the earlier workers (Iijima, 1884; Mattiesen, 1904) but we have definitely seen sperm in the uterus of *Dendrocoelum lacteum*. Even from the anatomical relationships in the genus *Planaria* it is obvious that there is no other alternative, as the undivided atrium is small and could not possibly accommodate the penis, which is therefore bound to go up the duct of the uterus. This was proved by Burr (1912) who succeeded in killing two animals actually during copulation.

The oviducts open into the posterior end of the duct of the uterus. In no way would it be possible for the sperm to enter them directly, for in addition to their extremely narrow lumina, the distal end of the penis of the partner extends up the duct of the uterus beyond their point of entry.

It is during copulation that the danger of self-fertilization is greatest. But the large penis, which carries at its apex the opening of the ductus ejaculatorius, extends on erection outside the genital atrium; this is therefore in itself sufficient to prevent the escape of sperm into the atrium of the same animal, with the consequent possibility of self-fertilization.

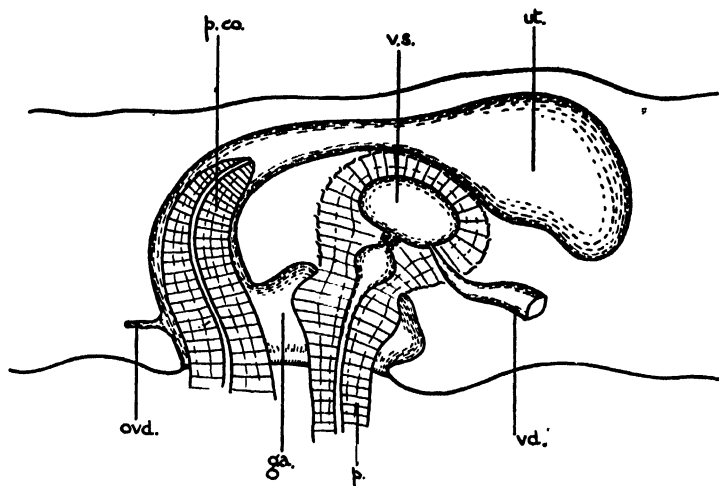
Text-fig. 2 (after Burr) illustrates these points. It should be noted that, as in most species, the penes are inserted simultaneously.

In other genera there seems to be a tendency to a reduction of the penis. This has led to modifications of the genital apparatus and even to the development of accessory organs. Two genera in which this reduction has taken place are *Dendrocoelum* and *Bdellocephala*.

Dendrocoelum lacteum.

In *Dendrocoelum lacteum* the genital atrium is divided into two parts, an inner and an outer. Into the outer cavity opens the stalk of the uterus and the muscular gland organ; in the inner lies the penis and the opening of the unpaired oviduct. It would seem peculiar that the penis and the oviduct should be in such close proximity, especially as the end of the

TEXT-FIG. 2.



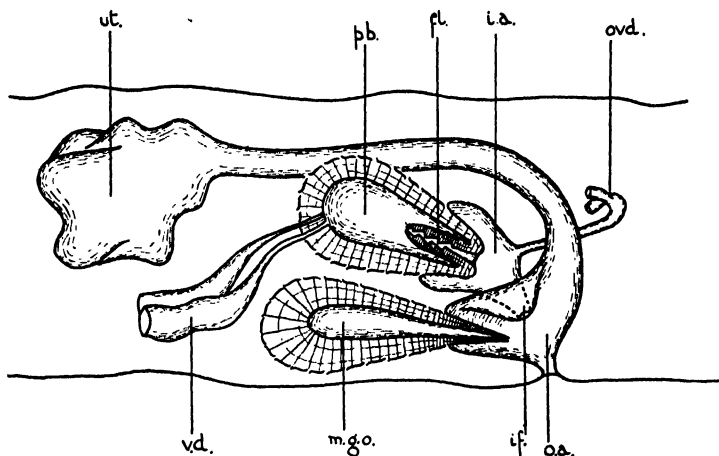
Schematic representation of the genitalia of *Planaria gonoccephala* during copulation. Simplified after Burr. For explanation of lettering see p. 479.

penis lies just below the opening of the oviduct (see Text-fig 3). At first sight it would seem inevitable that sperm should escape from the penis into the oviduct. But on closer examination the end of the penis is found to be equipped with a valve in the form of an introvert. The structure of this latter is peculiarly well adapted to its function. Our own observations on its structure confirm those of Iijima (1884). The inner side of the introvert is folded, the folds fitting in such a way that the lumen is completely obstructed, see photomicrograph (fig. 1, Pl. 1). This arrangement effectively prevents the exit of any sperm.

The other surface is smooth and, after eversion, would allow sperm to pass out of the penis readily. This introvert is usually called the 'flagellum'.

During copulation the flagellum is everted and passes through the inner atrial opening, which is in the form of an iris-like fold. The body of the penis fits closely into this opening, thus effectively sealing the inner atrial cavity with its oviduct. After

TEXT-FIG. 3.



Schematic representation of the genitalia of *Dendrocoelum lacteum* in a state of sexual quiescence; reconstructed from series of sections. For explanation of lettering see p. 479.

passing through the outer atrial cavity the flagellum protrudes out of the genital pore, and passes into that of the co-copulant finally entering the stalk of the uterus (Burr, 1912). This long extension of the penis effectively prevents the escape of any sperm even into the outer atrium of the same animal.

When the flagellum is inserted into the base of the stalk of the uterus, the corrugated surface, which is now the outer one, assists in making a close union between the two organs. This suggests that the fluid in the outer atrium may be harmful to the sperm, at least immediately after copulation, when some fresh water may have been introduced through the genital pore.

From the observed facts of the anatomical arrangement of the organs and the part they play in copulation, we think that the flagellum of the penis and the iris-like fold separating the inner from the outer atrium, can only be regarded as structures directly connected with the prevention of self-fertilization.

Bdellocephala punctata.

The most striking feature of the genitalia of this species is the reduction of the penis, which has no protrusible muscular papilla or ductus ejaculatorius. The part of the penis which is present corresponds structurally with the bulbos of the penis of other Triclad, and its lumen communicates with the genital atrium by a wide opening which has no sphincter and so cannot be closed. Ude (1908) in his description of the genitalia of this species finds a sphincter, but in none of our preparations is there any sign of such a structure. The muscular papilla of the penis, so conspicuous in the genus *Planaria*, is completely absent in *Bdellocephala*.

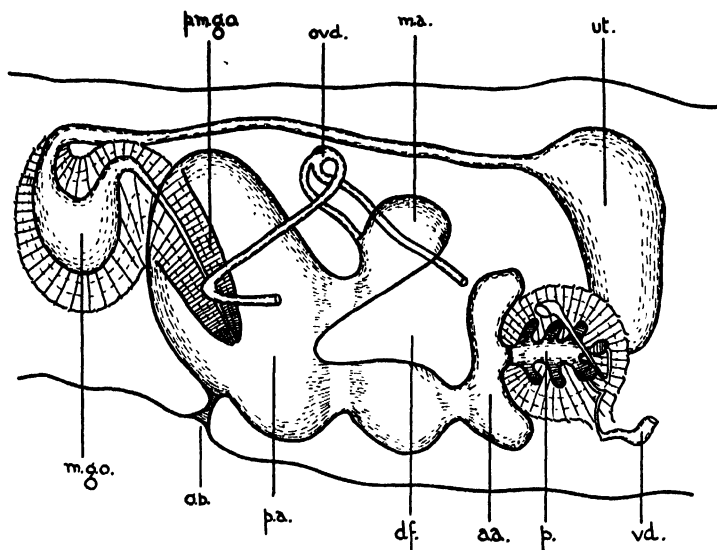
Sperm is not present in the male organ during sexual quiescence, but remains confined to the vasa deferentia. The mechanism which brings about this retention will be discussed later.

The atrium is divided into three parts by two large dorsal folds, each having a corresponding smaller ventral fold rising from the floor of the atrium towards it. In the most anterior of the compartments thus formed is situated the opening of the 'penis'; into the second or middle atrium opens the unpaired oviduct; the posterior atrium is put into communication with the exterior by the genital pore, while from its posterior wall projects the very muscular and extensible papilla of the muscular gland organ (see Text-fig. 4).

The body of the muscular gland organ lies embedded in the parenchyma posterior to the genital atrium. Like the papilla it is extremely muscular. Into its posterior end opens the stalk of the uterus, the lumen of which is very much narrower in *Bdellocephala* than in the other species of fresh-water Triclad: this condition, as we shall see later, is correlated with the method of copulation.

As we have seen, the structure of the penis is such that sperm which had found their way into its lumen could not be prevented from escaping into the atrium, with the consequent possibility of finding their way up the oviduct and causing self-fertilization. The mechanism for the prevention of the escape of sperm and

TEXT-FIG. 4.



Schematic reconstruction of the genitalia of *Bdellocephala punctata* in a state of sexual quiescence. From series of coronal and sagittal sections. For explanation of lettering see p. 479.

for its retention in the vasa deferentia is as follows: The vasa deferentia, which at the time of sexual activity are swollen with their content of sperm, are connected to the 'penis' only by extremely fine ducts, the course of which is difficult to follow owing to the complete occlusion of their lumina. This is occasioned by the presence of a special layer of circular muscles which surrounds these tubes and by contraction prevents the sperm from leaving the vasa deferentia, see photomicrograph (fig. 8, Pl. 1). This unusual musculature is found only in this species. In addition when the penis is in the position of rest

the tubes are bent in two places at acute angles, and this fact can be regarded as a further safeguard against the passage of sperm.

As will have been made clear by the anatomical description, the penis cannot carry out those functions which are usually associated with such organs; that is to say in this case the penis is so reduced as to be incapable of extrusion outside the genital pore and therefore cannot inject sperm into the partner. This being so, the narrowness of the stalk of the uterus in *Bdellocephala* is explained, for here there is no necessity for it to be wide, as it is in other species, where it has to accommodate the penis of the co-copulant.

We must also consider as connected with this condition of the penis in *Bdellocephala*, the fact that the stalk of the uterus opens through the cavity of the muscular gland organ, and not directly into the atrium as in other species. In the other Triclad's it has been shown that the uterus functions as a bursa copulatrix and there is no reason to suppose that different relationships hold good in *Bdellocephala*.

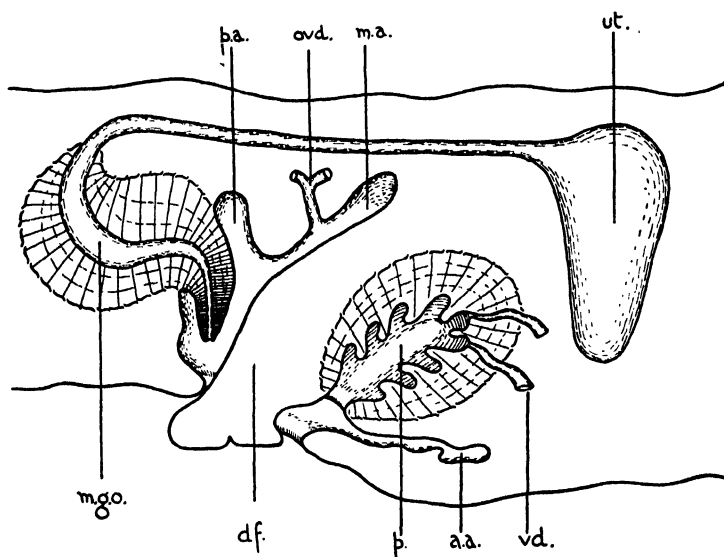
The natural and most obvious conclusion as to the actual process of copulation, is that the protrusible papilla of the muscular gland organ can be inserted into the cup-like penis of the co-copulant, and can suck out the spermatozoa. Then by muscular contraction it passes them up into the stalk of the uterus and so into the cavity of that organ (Ude, 1908).

The danger of self-fertilization is greatest at the moment of withdrawal of the papilla of the muscular gland organ of the partner at the end of copulation. From the diagram it would appear that the papilla would have to be withdrawn through the middle atrium into which open the oviducts, up which any escaping sperm would have a chance to penetrate.

Unfortunately, it has so far been impossible to kill two *Bdellocephala* while actually copulating, but an animal which was killed in a state of sexual activity showed how the dangerous possibility mentioned above is overcome by an alteration in the inter-relationships of the genital organs. The anterior dorsal fold, i.e. the one separating the anterior atrium with the penis from the middle one into which the oviducts

open, is remarkably extensible and can be protruded outside the genital pore; Text-fig. 5 showing this was reconstructed from serial sections (also see fig. 1, Pl. 1). This action puts the anterior (male) atrium into direct communication with the exterior, and at the same time alters the position of the penis,

TEXT-FIG. 5.



Schematic reconstruction of the genitalia of *Bdellocephala punctata* in a state of sexual activity. From a series of sagittal sections. For explanation of lettering see p. 479.

which is now drawn posteriorly towards the genital pore, and is rotated so that its opening faces postero-ventrally.

From the diagram it will be clearly seen that this extrusion of the anterior dorsal fold represents an arrangement by which the papilla of the muscular gland organ of the partner is guided into the cup-like penis at the onset of copulation. In addition, the angular bends in the muscular ducts joining the vasa deferentia to the penis are straightened and so the passage of sperm made possible. Further the anterior dorsal flap separates the anterior atrium from the middle atrium, and in this way

forms a mechanical barrier preventing any sperm, which may escape when the papilla of the co-copulant is withdrawn, from reaching the oviducts of the same animal.

SUMMARY AND CONCLUSION.

In various species of Triclad *Turbellaria* there are certain modifications of the genitalia which can best be interpreted as mechanisms for the prevention of self-fertilization.

In species of the genus *Planaria* the size and musculature of the penis and its manner of insertion are sufficient to prevent self-fertilization, but in other genera, notably *Dendrocoelum* and *Bdellocephala*, the penis has been reduced and self-fertilization is prevented by other modifications.

In *Dendrocoelum* the reduction of the penis is accompanied by the development of an introversible extension (the flagellum) at its end. When this flagellum is in the introverted position, which is the position of rest, it acts as a valve and prevents the escape of sperm. When extended during copulation, it assists by increasing the effective length of the penis in penetrating into the 'uterus' of the co-copulant, and prevents the escape of sperm into its own atrial cavity.

In *Bdellocephala* the penis is so reduced as to be no longer functional as such. The transfer of sperm is brought about by the muscular gland organ which has an extensible papilla which is inserted into the partner during copulation and which withdraws the spermatozoa from the cup-like penis. The possibility of self-fertilization occurring on the withdrawal of the muscular papilla is prevented by the extension of a flap from the dorsal wall of the genital atrium, which puts the male atrium into direct communication with the exterior.

Gelei (1924) suggested that self-fertilization did not occur because of physiological antipathy between the two sexual elements of the same animal. This is very unlikely, in view of the various mechanisms to be found in different species of fresh-water Triclads the sole function of which appears to be the separation of the sperm and ova of the same animal both before and during copulation.

Our thanks are due to Mr. J. T. Saunders for much help and advice in writing this paper.

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- Goetsch, W. (1925).—"Versuche über Selbstbefruchtung bei Planarien", 'Biol. Zentralblatt', Bd. 44, Heft 12.

The work of Goetsch only came to our notice after the main part of this paper had been printed. His experiments are essentially a repetition of those of Gelei, with the exception that *Planaria lugubris* was used as the experimental animal, and not *Dendrocoelum lacteum*. The results for *Planaria lugubris* were found to be the same as those obtained for *Dendrocoelum*, i.e. two animals which, when kept separately, had produced sterile cocoons, when allowed to pair both produced fertile cocoons.

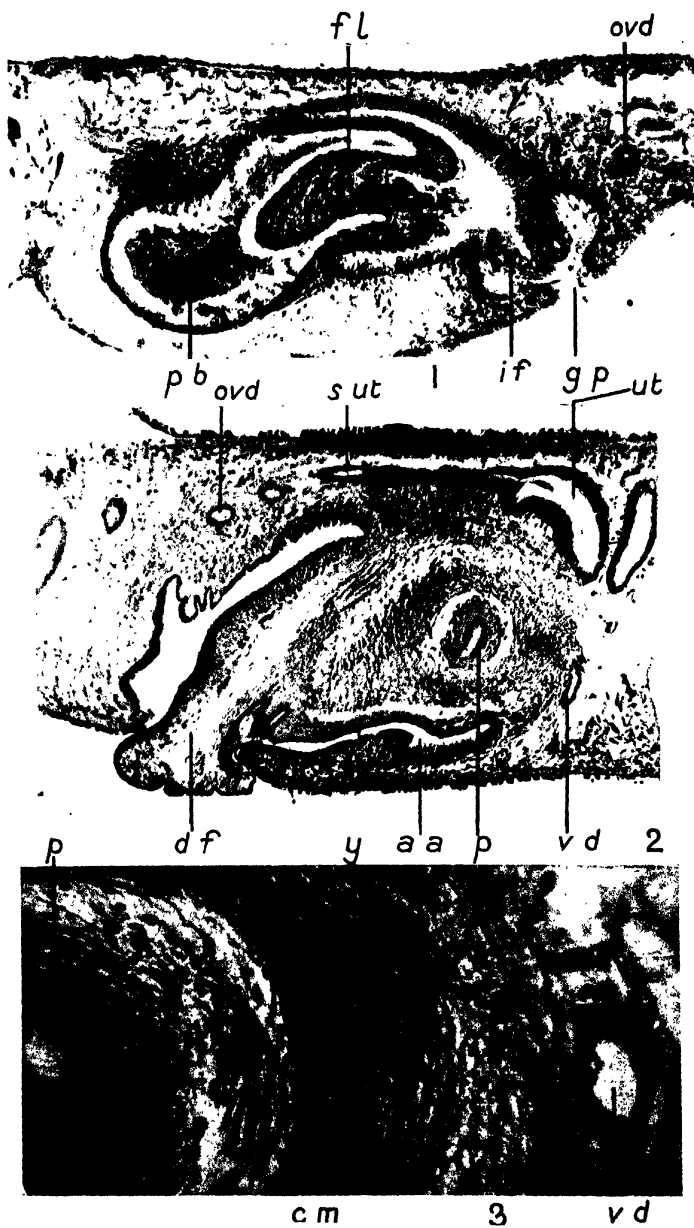
Goetsch makes no suggestions as to what mechanism is involved but merely says 'bei den Süßwassertricladen kommt eine Selbstbefruchtung nicht vor'. This confirms experimentally what has been said about *Planaria lugubris* in the earlier part of this paper.

EXPLANATION OF PLATES.

Fig. 1.—Sagittal section through the penis of *Dendrocoelum lacteum* showing the flagellum. $\times 50$.

Fig. 2.—Sagittal section through the genitalia of *Bdellocephala punctata* showing the extrusion of the anterior dorsal flap. $\times 50$.

Fig. 3.—Section transverse to the muscular duct joining the vas deferens to the penis in *Bdellocephala punctata*. $\times 250$.



On the Autonomic Nervous System of the Teleostean Fish *Uranoscopus scaber*.

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With 12 Text-figures.

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INTRODUCTION.

THE present study was undertaken with the object of making possible a comparison of the autonomic nervous system of teleostean fish with that of other classes of vertebrates. On a basis of his own and Gaskell's studies, Langley showed that the general visceromotor (autonomic) nerves of mammals may be divided into four sets or outflows (prosomatic, bulbar, thoracico-lumbar, and sacral), and that these can be grouped into two great physiological divisions, the sympathetic system being the thoracico-lumbar outflow, and the parasympathetic comprising the other three outflows. Further, it was shown that the autonomic systems of Birds (Langley, 1904) and of Amphibia (Langley and Orbeli, 1911 and 1912) are arranged on the same plan, and Langley (1903 *b*) assumes that the same may be said of fish but gives no references to support this assumption.

Although several books have appeared dealing with the autonomic system (Gaskell, 1915; Langley, 1921; Müller, 1924; Schilf, 1926; Stöhr, 1928; Kuntz, 1930) yet none of these gives much consideration to the system in vertebrates other than mammals. Gaskell, indeed, attempted some consideration of phylogeny and he states that 'in Teleosts, Ganoids, and Cyclostomes the sympathetic is not yet aggregated into chains but its cells are scattered in irregular groups about the veins'. This statement, though it may be true of Cyclostomes, is very far from true of the Teleosts, where the sympathetic chains are as well developed as in any mammal.

In fact previous studies of this system in fish do not enable us to compare it with that of other vertebrates, largely because they have been made with other methods and from other points of view. Chevrel (1887 and 1894) studied the macroscopic anatomy of the sympathetic system very thoroughly in a great number of different fish, and this study has been supplemented by various accounts based on the study of serial sections such as that of Herrick on *Menidia* (1899). As regards the physiology of the autonomic system of fish very little is known. Böhr (1894) brought some evidence to show that there is an 'antagonism' between the sympathetic system and the vagus in the

control of the contents of the air-bladder. v. Frisch (1911) and Wernøe (1925 and 1926) have studied the action of the sympathetic on the chromatophores. A few experiments have been made on the interesting questions of the innervation of the heart and the gut (see below). Müller and Liljestrand (1918) found that both the splanchnic nerves and the vagus produced motor effects on the stomach, and that there was no 'antagonistic' working.

The present paper contains an account of the macroscopic anatomy and course of the fibres of the sympathetic system of a single species of Teleost, *Uranoscopus scaber*. Special attention has been paid to the cranial region since it is here that the Teleost sympathetic shows most peculiarities. Various experiments dealing with the pupillary mechanism will be described in another paper, to which it will be necessary occasionally to refer. It was found that the innervation of the iris muscles differs markedly from that of mammals in that the sympathetic nerves constrict the pupil whereas the fibres in the oculomotor dilate it (Young, 1931).

MATERIAL.

Uranoscopus scaber was chosen among the host of Teleosts available chiefly on account of the ready accessibility of the sympathetic chains. In most bony fishes these lie in a narrow groove below the vertebral column and in many cases they are covered with a dense layer of pigment. In *Uranoscopus* the two chains run widely separate, they are easy of access and almost free of pigment.

Uranoscopus lives buried in the sand, in rather shallow water, with the body completely hidden and only the two eyes protruding. The eyes are on the top of the head and can be pushed right out of their sockets or, if necessary, rapidly withdrawn. The mouth opens upwards; attached to the lower jaw is a vascular strip which darts in and out and serves as a bait for small fishes, &c., which are swallowed whole.

In accordance with this mode of life the structure of the fish departs somewhat from that of a normal Teleost. The body is flattened dorso-ventrally, the mouth is very large and there is

no swim-bladder. These modifications do not in any way affect the present investigation. The absence of a swim-bladder is an advantage (unless that organ itself is to be studied) and so is the flattened shape of the body, which makes dissection easier than in the normal Teleost. No attempt has been made to give a complete account of the autonomic system of all Teleosts; the aim has been to discover the fundamental nature and organization of this part of the nervous system in fish, and this can best be done by description and experiments on single forms. Our knowledge of the mammalian autonomic system is based not so much on comparative accounts as on the close study of one or two laboratory animals. It is unsafe, however, to generalize too widely or to apply results obtained from one species to a whole order; further research may reveal other conditions in other fish.

Uranoscopus has the further advantage of being very common at Naples where the present investigation was carried out.¹ It lends itself well to experiment, especially in that it lies quietly on the bottom so that the eyes can be readily observed. Unfortunately the time of survival of this fish in the aquarium is limited to about three weeks.

ANATOMY OF THE SYMPATHETIC SYSTEM AND COURSE OF THE FIBRES.

1. Methods.

(a) *Osmic Acid*.—The anatomy of the sympathetic has been studied chiefly by means of dissections under the low-power binocular microscope, the nerves being stained with osmic acid. This not only helps the dissection by darkening the nerve-trunks but also makes possible a finer analysis under the microscope, since the medullated fibres are beautifully

¹ The work was done at the Oxford table at the Naples Zoological Station during tenure of the Christopher Welch Scholarship and of a Senior Demyship from Magdalen College, Oxford. I should like to thank the bodies concerned for these grants and also Professor R. Dohrn and all the staff of the Zoological station for their hospitality and assistance. I am also greatly indebted to Professor Goodrich for suggestions and for reading the manuscript of this paper.

stained in black, whereas the non-medullated fibres and ganglion cells become brown. Probably all nerves darken to some extent with osmic acid on account of the fatty substances contained in the axons, but no confusion is possible between the distinct, black, medullated fibres and the imperfectly stained non-medullated nerves in which the fibrous structure can only vaguely be made out.

Some doubt seems to exist as to the distribution of medullated fibres in the sympathetic system and hence as to the value of osmic acid in the study of these nerves. Gaskell (1886) at first supposed that all the preganglionic fibres running out in the white rami communicantes from the central nervous system to the sympathetic system were medullated and that all post-ganglionic fibres were non-medullated. However, it has since been shown (see Langley, 1921) that some pre-ganglionic fibres lose their sheaths before they terminate, and that many post-ganglionic fibres are medullated. For instance the fibres of the short ciliary nerves are medullated in all vertebrates; Langley (1896) found many medullated fibres in the lumbar and sacral grey rami in the cat and in the dog but not in the rabbit; however, Kuntz and Farnsworth (1928) have produced some evidence that these are sensory fibres, running to the sacral plexus. Langley and Orbeli (1911 and 1912) proved the presence of medullated post-ganglionics in the head of the toad. Dogiel (1908) noticed that many post-ganglionic fibres acquire a sheath after they have run some distance from the cell. Ranson and Billingsley (1918) came to the conclusion that the great variation in the number of medullated fibres in the branches of the superior cervical ganglion showed that 'the way in which the myelin sheaths are distributed seems to be entirely accidental' (p. 381). Langley (1903*b*, 1921) states that, 'in vertebrates other than mammals the rami communicantes of the spinal nerves consist wholly, or almost wholly, of medullated fibres, grey rami communicantes are not present'. This may be true of birds and amphibia but, as will be shown in this paper, it certainly does not apply to fish.

It must be concluded, then, that the presence or absence of a medullary sheath is no sure evidence of the functional position

of fibres in the autonomic system, but that in nearly all cases non-medullated fibres are post-ganglionic, although the converse does not by any means hold. Osmic acid can therefore be of considerable help in the analysis of the sympathetic of those forms in which the post-ganglionics are non-medullated; but any conclusions based on its use must be accepted with reserve until checked by other methods.

If the animal is fresh, weak solutions of osmic acid can be used (0.5 per cent. or weaker). If it is applied to the nerves *in situ* it should be allowed to act for 20 minutes or more. It is important that all the surrounding tissues should be cleared away. If necessary several applications can be made. The nerves will also blacken with osmic acid after fixation for a short time in formalin, but in this case stronger solutions (up to 2 per cent.) must be used. Very good results are obtained by first cutting out the fresh nerves and then staining in weak osmic acid. In all cases the pieces must be well washed and they can then be mounted in glycerine or Apathy's syrup and studied under the microscope. Most of the accompanying figures are camera lucida drawings of such preparations.

(b) Weigert Method.—In order to confirm the results obtained from the study of whole mounts of ganglia stained in osmic acid, serial sections were made of the more important regions and stained by a modified Weigert method, as suggested by Gozzano (1929). The nerves were fixed in formalin, post-chromed for seven days at 35° C., and then embedded in paraffin and cut into serial sections 15 μ thick. After mordanting in 4 per cent. iron alum the sections were stained in haematoxylin at 35° C. and differentiated after Pal. With this method even the finest sheaths are stained an intense blue while the non-medullated fibres can be traced as brown bundles.

(c) Other Methods.—Several silver methods were tried without success, namely: Cajal (several methods), Bielschowsky, Bielschowsky-Gros, and Schultze methods. These were being used at the same time with great success on Selachian and other material, but Teleosts are known to be particularly refractory to silver stains.

A few methylene blue preparations were made, and this would

seem to be the best method for a study of the histology of the sympathetic of fish (see Huber, 1900).

2. Divisions of the Sympathetic System.

The sympathetic chain of *Uranoscopus* may be considered to extend on each side from the oculomotor nerve to the tip of the tail and to bear a ganglion in each cranial and spinal segment. For convenience it may be divided into cranial and spinal regions. The spinal region may be further subdivided into anterior and posterior trunk and caudal regions. The division between the two parts of the trunk sympathetic is taken to be at the level at which the two posterior cardinal veins come together in the middle line behind the large m.m. retractores dorsales arcuum branchialum, at which point the two sympathetic chains begin to be connected by transverse commissures.

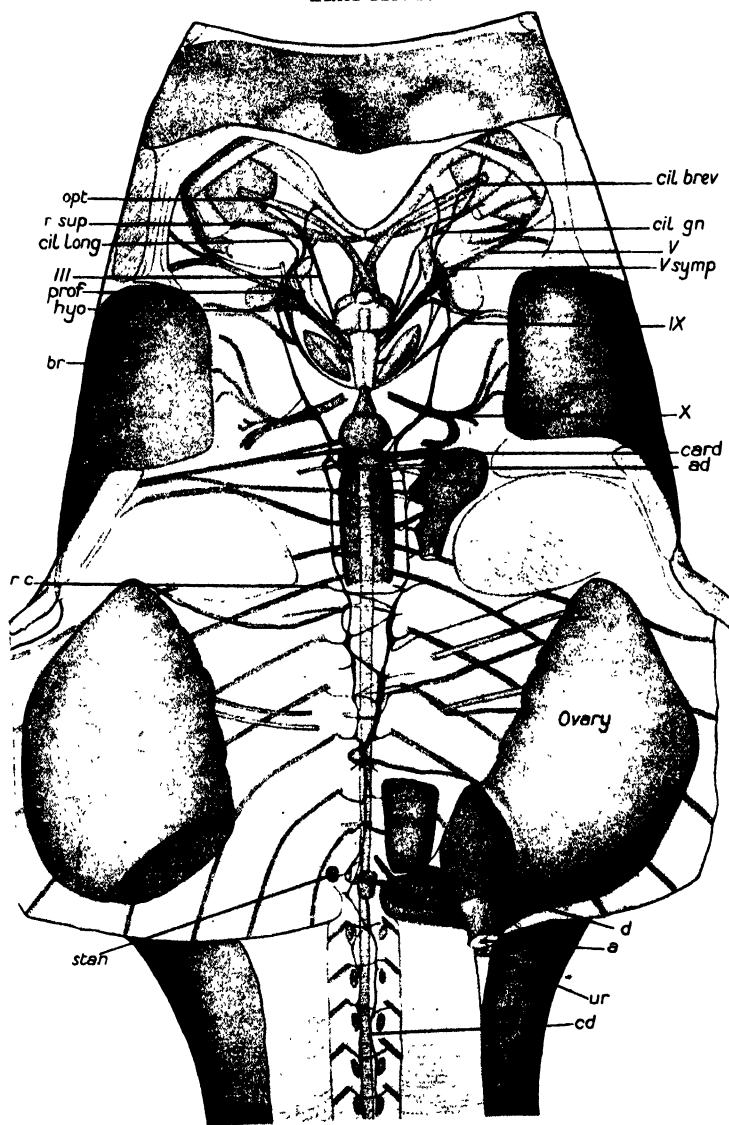
In this account of the sympathetic, the plan of a typical spinal segment will first be outlined and then the various ganglia described in detail, beginning at the first spinal segment and from here working first backwards and then forwards.

3. Plan of the Ganglia.

The sympathetic ganglia of the normal spinal segments are arranged on the same plan as those of a mammal. In each segment there is, on each side, a ganglion placed on the chain and communicating with the spinal nerve by a ramus communicans which is double and comprises a white ramus of pre-ganglionic fibres running out from the central nervous system via the ventral root, and a grey ramus of non-medullated fibres which run back and out along the spinal nerves to be distributed to the blood-vessels, muscles of the fin rays (Chevrel, 1887), and to the chromatophores. Such ganglia are seen in Text-figs. 2, 3, and 5, and diagrammatically in Text-fig. 12. The white and grey rami often run separately and, as in mammals, there is considerable variation in the actual number of rami per segment, there being sometimes two or more grey rami running separately to the spinal nerve.

I have not sufficient preparations to say for certain that medullated fibres never run in the recurrent grey rami, but

TEXT-FIG. 1.



General view of a dissection of the sympathetic nervous system of female *Uranoscopus*. Ventral view. About life size.

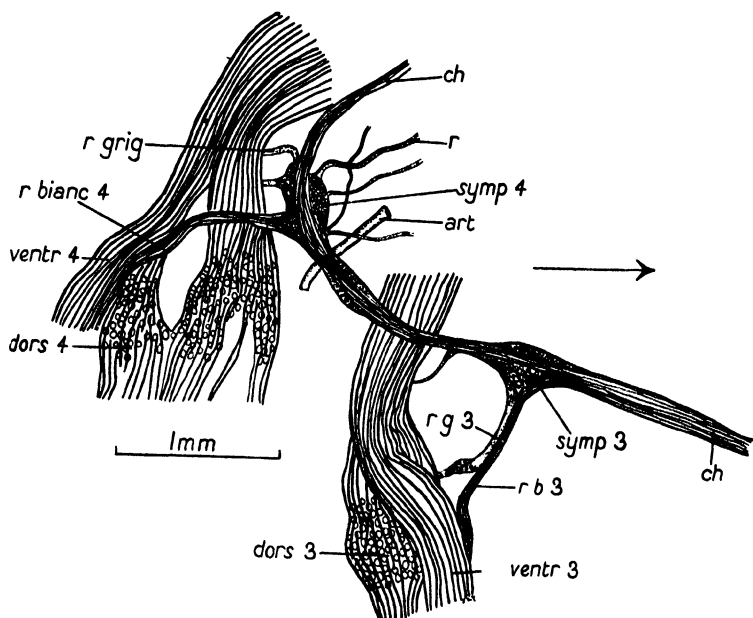
LIST OF ABBREVIATIONS USED IN THE TEXT-FIGURES.

a., anus; *ad.*, adrenal tissues; *art. ov.*, ovarian artery; *art. seg.*, segmental artery; *bl.*, urinary bladder; *br.*, gill-chamber; *carg.*, cardiac nerve; *cd.*, caudal sympathetic; *ch.*, sympathetic chain; *cil. brev.*, short ciliary nerve; *cil. gn.*, ciliary ganglion; *cil. long.*, long ciliary nerve; *d.*, mesonephric duct; *dil.*, m. dilatator pupillae; *dors. 1, 2, &c.*, dorsal root ganglia; *f.*, single large medullated fibre; *g. Gass.*, Gasserian ganglion; *g. VII.*, geniculate ganglion; *hyo. VII.*, n. hyomandibularis facialis; *hyp.*, hypoglossal nerve; *l.*, lens; *lig.*, ligament; *loop*, loop of sympathetic round hyomandibular; *n. orb.*, nerve to wall of orbit; *n. ov.*, ovarian nerve; *n. sp.*, spinal nerve; *n. sph.*, nerve to urinogenital sphincters; *n. splanch.*, splanchnic nerve; *o. inf.*, m. obliquus inferior; *op. v.*, ophthalmic vein; *opt.*, optic nerve; *p.*, pupil; *p. op. p.*, portio ophthalmicus profundus (?); *prof.*, profundus ganglion; *r.*, nerves to adrenal tissue; *r. III.*, oculomotor root; *r. VII.*, facialis root; *r. ant.*, m. rectus anterior; *r. b.* and *r. bianc.*, white ramus communicans; *r. branch.*, branchial branch of vagus; *r. comm. V.*, *1st sp.*, &c., rami communicantes; *r. g.* and *r. grig.*, grey rami communicantes; *r. inf.*, m. rectus inferior; *r. post.*, m. rectus posterior; *r. sup.*, m. rectus superior; *r. visc.*, visceral branch of vagus; *rad. brev.*, radix brevis ad ganglion ciliare; *rad. long.*, radix longa ad ganglion ciliare; *rad. sens.*, radix sensoria ad ganglion ciliare; *rad. symp.*, radix sympathica ad ganglion ciliare; *rec.*, recurrent fibres in rad. brev.; *sph.*, m. sphincter iridis; *sp. n.*, spinal nerve; *Stan.*, corpuscle of Stannius; *symp. V, 3 sp.*, &c., sympathetic ganglia; *tr.*, transverse sympathetic commissure; *u.* and *ur.*, urinogenital aperture; *ventr. 3, 4, &c.*, ventral roots; *w.*, nerve to ophthalmic artery; *x.*, turning-point of fibres; *y.*, nerve from sympathetic to oculomotor; *z.*, nerve to artery; *III.*, oculomotor nerve; *IV.*, trochlear nerve; *V.*, trigeminal nerve; *VI.*, abducens nerve; *VII.*, facial nerve; *IX.*, glossopharyngeus nerve; *X.*, vagus nerve; *V. mand.*, r. mandibularis V; *V. op. sup.*, r. ophthalmicus superficialis V; *V. symp.*, trigeminal sympathetic ganglion; *VII. pal.*, r. palatinus facialis; *1st symp. gn.*, &c., sympathetic ganglia.

certainly, in contradiction to the statement of Langley quoted above (p. 495), the majority of the fibres are non-medullated and constitute a true grey ramus. In this respect *Uranoscopus* resembles many mammals and differs from Birds and Amphibia (in which the recurrent rami are composed of medullated fibres). In Selachians, as will be shown in a future paper, there are no recurrent rami at all, the ramus communicans consists solely of pre-ganglionic connector elements.

In many of the rami a curious condition is seen in which one of the somatic fibres of the spinal nerve (recognizable by its much larger diameter) turns out from the nerve with the white ramus and runs with it for some distance before turning round into the grey ramus which it follows back into the spinal nerve

TEXT-FIG. 2.



Right third and fourth spinal roots and sympathetic ganglia; seen from the ventral side. Cam. luc.

(Text-fig. 3). Presumably these are fibres which have been pulled or pushed out of position by the outgrowing sympathetic fibres.

The pre-ganglionic fibres do not by any means always end in the ganglion of the segment in which they run out; many run forwards and backwards in the chain and may cross over to the chain of the opposite side. There is little evidence to show whether all the post-ganglionic fibres run out in the segment in which they arise. Langley was of the opinion that they

do not usually run forwards or backwards in the chain. In *Uranoscopus* there are certainly some non-medullated fibres in the chain between the ganglia (this is best seen in Weigert preparations) and probably the post-ganglionic fibres

TEXT-FIG. 3.



Photomicrograph of the point of junction of a ramus communicans and spinal nerve. Magnified 90 \times .

run in the chain for at least a short distance; they may also cross in commissures to the opposite side (see Text-fig. 5).

4. First and Second Spinal Sympathetic Ganglia and Splanchnic Nerves.

There is no separate hypoglossal nerve in *Uranoscopus*. The first nerve to leave the vertebral column behind the vagus is a complete spinal nerve with a spinal ganglion. This gives off a ventral branch which runs round below the pericardium to innervate the hypobranchial musculature and represents the hypoglossal nerve.

The first two spinal nerves run out close together; together with a part of the third spinal nerve they form the brachial plexus.

The sympathetic chains at this level lie close to the sides of the vertebral column separated from the corresponding nerves by several muscles and ligaments attached to the back of the skull. The rami communicantes are long; usually there are two of them and two ganglia, but the latter often fuse and there may be only one ramus communicans. These first rami communicantes are composed almost entirely of non-medullated fibres, presumably post-ganglionic; they contain only a few medullated fibres and these are probably sensory. The majority of the pre-ganglionic fibres for the ganglia of these segments run out more posteriorly and thence forwards in the chain.

On the right hand side the large splanchnic nerve springs from these first two ganglia; on the left there is no splanchnic but a large commissure runs right across ventrally to the aorta to join the right first spinal sympathetic ganglion. Chevrel (1887) could not find this commissure in *Uranoscopus* although it is present in other Teleosts; possibly in some individuals there is a separate splanchnic nerve on the left side and no commissure, but I have never seen such an arrangement.

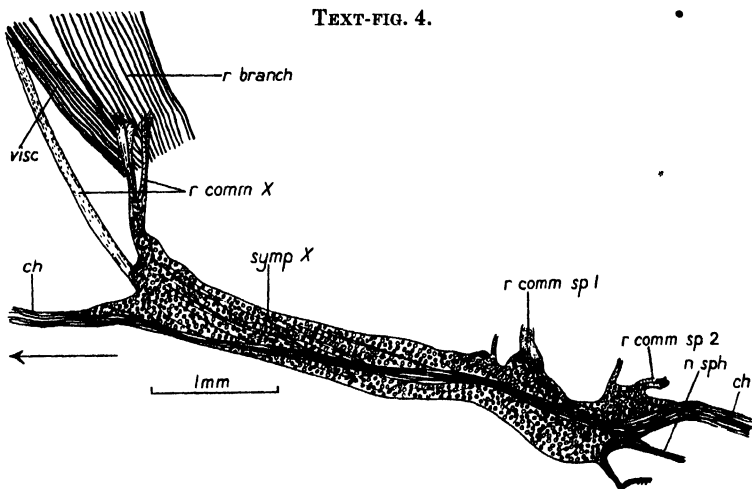
The splanchnic nerve contains non-medullated fibres and a large bundle of medullated fibres which can be seen to turn out into it from the chain (Text-fig. 4). These medullated fibres probably include sensory elements and pre-ganglionic fibres for the splanchnic and other sympathetic ganglia along the gut. The further course of the splanchnic nerves has not been traced; Chevrel reports that in other Teleosts sympathetic fibres run to the stomach, intestine, liver, pancreas, &c. There is no other splanchnic nerve behind the first; the hind part of the gut, if it receives sympathetic fibres, must take them from in front.

5. Cardiac Nerves.

Careful search was made for nerves from the anterior abdominal sympathetic ganglia to the heart, but none were found. Previous observers (Stannius, 1849; Chevrel, 1887) have also been unable to find direct connexions between the sympathetic

and the heart; nor have physiologists found any accelerator fibres, the vagus inhibits the heart beat both in Selachians and Teleosts (Brücke, 1925; Clark, 1927; Izquierdo, 1930). If such fibres existed they would probably run along the walls of the ductus Cuvieri, but the only nerve found in this position is the cardiac branch of the vagus. The latter (*card.*, Text-fig. 1) springs by several roots from the visceral branch of the vagus

TEXT-FIG. 4.



Left vagal and 1st and 2nd spinal sympathetic ganglia; ventral view.
Cam. luc.

and runs round ventral to the gut in the loose tissue just anterior to the ductus Cuvieri. Farther on it penetrates the wall of the ductus and runs on the inner side as far as the sinus venosus where it splits up into several smaller branches most of which converge on the sinuauricular opening, around which there is a complicated plexus, containing many nerve-cells. The whole arrangement very much resembles that of a Selachian.

The cardiac nerves consist of medium-sized medullated fibres, of which there are about twenty on each side. It is possible that these nerves contain sympathetic fibres which have joined the vagus via the ramus communicans. This is apparently the course taken by the sympathetic fibres to the heart in Am-

phibia (Gaskell, 1886; Langley and Orbeli, 1911). However, the ramus communicans to the vagus consists only of non-medullated fibres, and since the cardiac nerve contains only medullated fibres it is unlikely that any of them are from the sympathetic. This cannot be taken as absolute proof since it is well known that post-ganglionic fibres may acquire a sheath after running for some distance (see p. 495). The question could be decided by cutting the vagus and allowing time for degeneration. *Uranoscopus* would probably also be very suitable for determining whether stimulation of the sympathetic has any effect on the heart.

6. Sympathetic Ganglia of the Anterior Trunk Region.

Passing backwards from the first two spinal sympathetic ganglia the chain bends mesially across the ventral face of the head kidney to the third abdominal sympathetic ganglion (Text-fig. 2). This lies close to the ventral face of the third spinal ganglion so that the rami communicantes are very short. The white ramus is particularly large; it can be seen in the figure emerging from the ventral root. Only a few of its fibres end in the sympathetic ganglion of the same segment; the majority of them turn forward in the chain to supply the more anterior ganglia. The further course of these fibres is traced on p. 513.

The fourth spinal sympathetic ganglion lies close behind the third, and like it lies close to the corresponding spinal ganglion. The white ramus divides into equal parts running forwards and backwards in the chain.

The fifth and sixth ganglia are quite small, as are their rami communicantes. The pre-ganglionic fibres seem to end in the ganglion of the same segment or to turn backwards in the chain. From all these anterior sympathetic ganglia many small nerves pass to the tissue which surrounds the front end of the cardinal veins. In this region lie the tissues which Giacomini (1902, 1908, 1920, &c.) has shown to be homologous with the interrenal and suprarenal of Selachians, that is to say, with the adrenal glands of Tetrapods. It is to be expected that this organ

would receive a plentiful supply of nerves from the sympathetic. These nerves contain medullated as well as non-medullated fibres, the former being probably pre-ganglionic fibres for the ganglion cells which lie among the glandular tissues (Giacomini).

The sympathetic chains in this region run close to the vertebral column, mesial to the cardinal veins. They are widely separated by the retractor muscles of the branchial arches and are not joined by transverse commissures. They frequently divide round the segmental arteries.

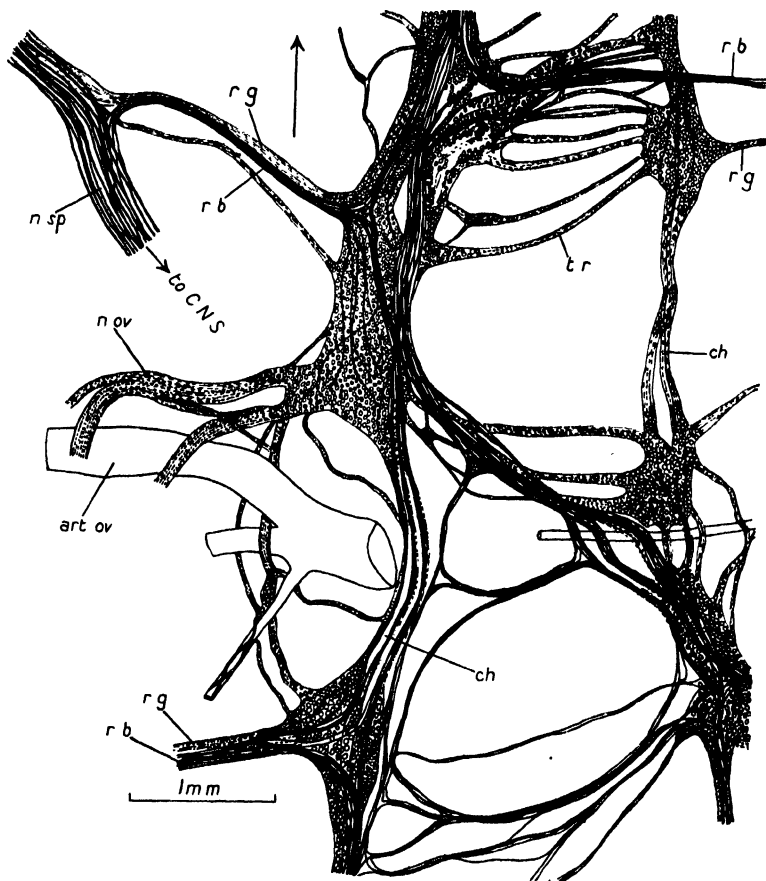
7. Sympathetic Ganglia of the Posterior Trunk Region.

Behind the branchial retractor muscles the two cardinal veins come together in the middle line and run as a single vein between the excretory portions of the mesonephros. The sympathetic chains also approach the middle line and transverse commissures cross between them. The ganglia themselves (Text-fig. 5) are much less regular than those more anteriorly; they are flattened dorso-ventrally between the dorsal aorta and the cardinal vein and tend to be diffuse and spread out along the cords. There is always a pair of double rami communicantes in each segment; generally there is a ganglion where the ramus enters the chain. Often the chain and ganglia are reduced on one side, the fibres running in the opposite chain.

The first commissures behind the retractor muscles consist of medullated fibres which run down from one chain and up into the opposite one. They are probably commissural fibres for the ganglia which lie in front. The succeeding commissures are very numerous and irregular (see Text-fig. 5). They contain both medullated and non-medullated fibres. In some cases the entire bundle of pre-ganglionic fibres from a white ramus may be seen to pass through the ganglion of its own side and across to the opposite chain in which it may turn forwards or backwards. The presence of transverse commissures in the sympathetic is common in all Tetrapods, especially in the posterior part of the body. Wernøe (1925) has shown that in fish the pre-ganglionic fibres which control the expansion of the chromatophores may cross from one side to the other.

Passing backwards between the kidneys the two chains approach one another and eventually fuse and pass as a single

TEXT-FIG. 5.



Sympathetic chains in posterior trunk region; showing the origins of the ovarian nerves. Cam. luc.

cord to the end of the abdomen. Where it passes into the haemal canal the cord again becomes paired.

The rami given off from the sympathetic in the posterior trunk section are chiefly those to the kidneys, gonads, and urinary

bladder, besides the recurrent rami to the spinal nerves and certain other isolated branches, composed of non-medullated fibres, which plunge into the muscles, usually in company with an artery. These presumably correspond to the 'dorsal rami' found by Andersson (1892) in *Urodeles*, by Chevrel (1887) in *Teleosts*, and by Jenkin (1928) in *Lepidosiren*. Jenkin has attempted a comparison of types of sympathetic, partly on a basis of these ramuli. Such a comparison is of doubtful value in view of our ignorance of the course of the fibres and considering the plasticity of the sympathetic. Small non-medullated nerves are apt to be given off to accompany all arteries.

8. Innervation of the Urinogenital System.

The excretory mesonephroi consist of short flattened strips of tissue lying near the middle line but separated by the median posterior cardinal vein. The mesonephric ducts are very short (see Text-fig. 1) and open out into a large urinary bladder. This is a mesodermal structure, derived from the expanded bases of the mesonephric ducts, it is not homologous with the cloacal bladder of *Tetrapods*.

The gonads, both ovaries and testes, are hollow sacks. The genital and mesonephric ducts do not open separately in *Uranoscopus*, as they do in the majority of *Teleosts*, but together, into a short urinogenital sinus opening just behind the anus (Text-fig. 6).

The large genital nerves arise from the sympathetic of the posterior abdominal region and run round dorsally to the kidney and along the mesovarium (or mesorchium) to the gonad. They always accompany the genital arteries, of which there are usually three or four pairs. The largest arteries and nerves are at about the middle of the length of the mesonephros and at this level the sympathetic ganglia are very large (see Text-fig. 5). The great majority of the fibres in the genital nerves are non-medullated and therefore probably post-ganglionic.

The walls of the ovaries have been observed to make spontaneous movements, but stimulation of the sympathetic in this region was not observed to have any effects on the gonads.

The urinary bladder is also innervated from the sympathetic system, the nerves passing with the vesicular arteries round the extreme hind end of the mesonephros. Small branches are given off to the corpuscles of Stannius which lie embedded in the mesonephric tissue (*stan.*, Text-fig. 1). Before reaching the bladder the nerve divides, and at the point of bifurcation there are small collections of ganglion cells. From these ganglia the nerves run on to the walls of the bladder in which they form a plexus in which can be seen numerous ganglion cells. Other fine nerves run directly to the mesonephric duct (and sphincters?) around which they form a fine plexus (Text-fig. 1).

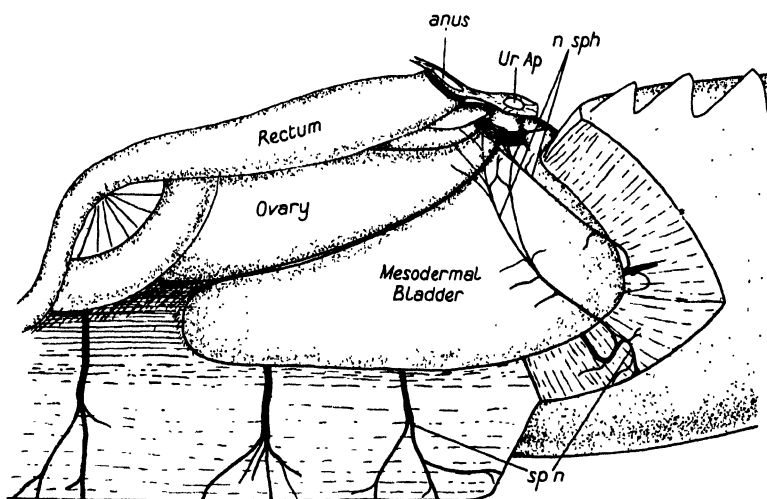
The vesicular nerves, unlike those to the gonads, contain many medullated fibres which can be seen to run out from the main tract of medullated fibres in the sympathetic chain. Some of these fibres end in the ganglia along the course of the vesicular nerves and others in the plexus in the wall of the bladder.

The only parts of the urinogenital apparatus to receive fibres other than those from the sympathetic system are the openings of the bladder and gonads into the median urinogenital sinus. These openings are surrounded by sphincters which are innervated by branches of the neighbouring spinal nerves (Text-fig. 6). Although these sphincter nerves pass close to the urinary bladder no fibres could be seen passing to the latter; neither could any branches be traced to the anal sphincter although there are several ramuli running in that direction. These nerves to the sphincters may be homologized with the nervus pudicus of mammals; the muscles which they innervate are striped muscles, probably derived from the myotomes and not from the lateral plate.

As stated above (p. 502), there is only one anterior, splanchnic nerve to the gut and no visceral branches have been seen arising from the sympathetic other than those described. There is nothing corresponding to the sacral (parasympathetic) outflow of fibres to the lower intestine which is found in Tetrapods. The vesicular nerve of *Uranoscopus* cannot be homologized with the pelvic nerve of mammals since the bladders are of different origin in the two cases.

The arrangement of the visceral nerves of *Uranoscopus* seems to be definitely simpler than that of Tetrapods. The gut receives fibres from the vagus and from the sympathetic (in two outflows, one running with the vagus and one in the

TEXT-FIG. 6.



Semi-diagrammatic view of the urinogenital and anal apertures of a female *Uranoscopus*.

splanchnic nerves). Whether there is antagonistic action of these two systems in the gut is not known and remains a very interesting subject for investigation. Bottazzi (1902) could find no such antagonism in Selachians, and Müller and Liljestrånd (1918) found that both vagus and splanchnic nerves produce only motor effects in the gut, both of Selachians and Teleosts.

The whole of the gonads and the mesonephros and its derivatives, the kidneys and mesonephric bladder, are innervated from the sympathetic only, and there is apparently no other, parasympathetic, innervation. The sphincters at the end of the urinogenital ducts are innervated by twigs from the spinal nerves. This again is a simpler arrangement than that of Tetrapods in which the organs of the urinogenital system

receive a double innervation—from the vagus and from the sympathetic.

9. Caudal Sympathetic System.

After giving off the nerves to the bladder the single sympathetic chain divides into two chains as it runs into the haemal canal and these pass backwards on either side of the caudal vein. Throughout the tail the cords run on either side of the aorta and bear small ganglia in every segment, each connected with the spinal nerve by means of white and grey rami communicantes. The chains are connected by one or more transverse commissures in every segment. Frequently the cord is more developed on one side than the other and it may become single for several segments.

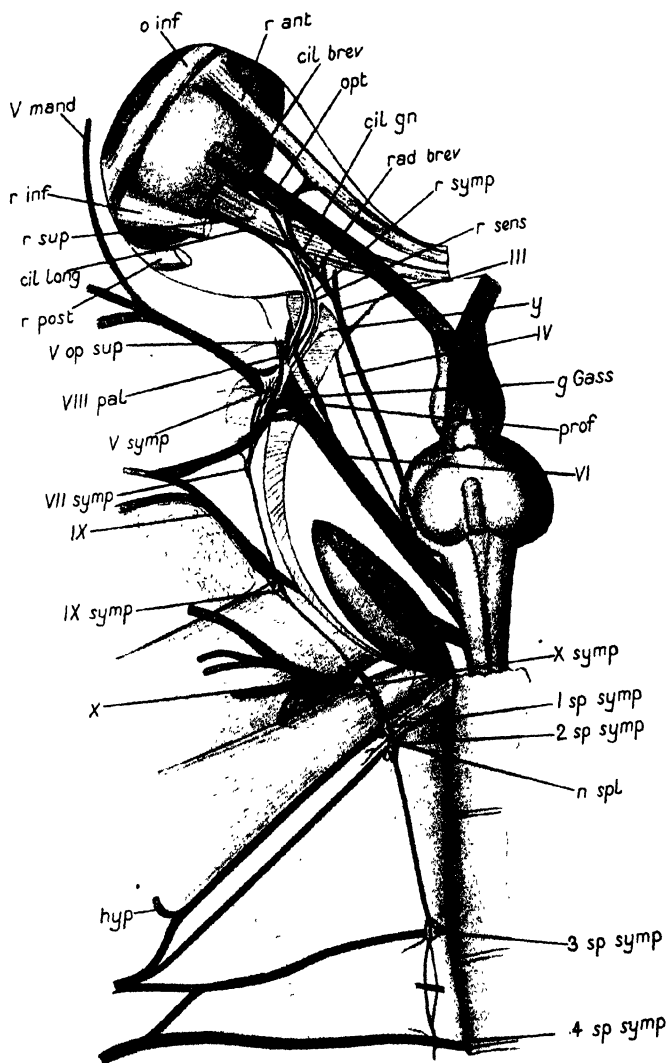
A large bundle of medullated fibres runs backwards into the tail from the more anterior sympathetic. Possibly these are fibres concerned with the innervation of the chromatophores. v. Frisch (1911) has shown that all the pre-ganglionic fibres which innervate the latter run out in a few segments in the middle of the body and thence forwards and backwards in the sympathetic chain. Wernøe (1925) states that stimulation of the sympathetic at the extreme hind end of the tail causes colour changes over the whole body, which he takes to be mediated by these 'Präganglionäre Universalneuronen'.

10. Cranial Nerves.

Before describing the cranial sympathetic a short account must be given of the cranial nerves. There is nothing remarkable about the eye-muscle nerves; the eye-muscles themselves enter the skull and run in a short myodome to meet in the middle line (Text-fig. 7).

One of the peculiarities of the cranial nerves is the presence of a separate profundus ganglion. The profundus nerve typically, as in some Selachians, has dorsal and ventral branches like any other dorsal root. The dorsal branch includes the portio ophthalmica profundi or ramus frontalis (which usually joins the ramus ophthalmicus superficialis) and part of the long ciliary nerve. The ventral branch includes the ramus ophthal-

TEXT-FIG. 7.



Drawing of a dissection of the anterior part of the sympathetic system
Ventral view. About twice natural size.

micus profundus (ramus nasalis) and part of the radix longa ad ganglion ciliare.

In all bony fish the profundus is reduced. In *Polypterus*, *Amia*, and *Lepidosteus* (Allis, 1897; Norris, 1925) the ramus nasalis is still present as a separate nerve, but in Teleostei it seems to be completely lacking, although other parts of the profundus persist. The profundus ganglion generally becomes fused up with the Gasserian ganglion, *Menidia* (Herrick, 1899), but it remains separate in *Trigla* (Stannius, 1849), and in *Scomber* (Allis, 1903). In *Menidia* Herrick found that part of the Gasserian ganglion was separate from the rest and associated with a separate lobe of the sympathetic ganglion. From this joint mass arise the long ciliary nerve and the radix longa and he considers that this part of the ganglion represents all that is left of the profundus, both ramus frontalis and nasalis being absent.

In *Uranoscopus* (Text-figs. 7, 8, 9) the profundus and trigeminus roots are separate right back to their origin from the brain although the profundus is very thin and is bound up loosely with the front edge of the trigeminus. Just before reaching the skull wall it separates off and bears the small profundus ganglion, which is quite separate from the Gasserian ganglion. Two nerves arise from the profundus ganglion: a very thin branch (*p.op.p.*) comes off ventrally and passes round to join the trigeminus just before it passes through the skull wall. It seems possible that this represents the ramus frontalis (portio ophthalmica profundus) which joins the ramus ophthalmicus superficialis in Selachians.

The larger branch of the profundus nerve passes out through a foramen of its own and emerges in the palatine canal where it joins the radix sympathica with which it runs partly as the long root to the ciliary ganglion and partly as the long ciliary nerve (see diagram, Text-fig. 8).

11. Vagal, Glossopharyngeal, and Facial Sympathetic Ganglia.

Passing forwards from the first spinal sympathetic ganglion the chain bends outwards to the point of emergence of the vagus

nerve. The sympathetic in this region consists of a broad strip of ganglion cells through the middle of which runs a large bundle of medullated fibres (Text-figs. 4, 7). These fibres are those which are seen to run out in the white rami communicantes of the third and fourth spinal segments and to turn forwards. Some fibres branch off from this tract to end in the first two spinal and in the vagal sympathetic ganglia, while a large bundle turns out in the splanchnic nerve; the majority, however, run on right through all these ganglia.

The ramus communicans to the vagus is very large and comprises a separate branch to each of the branchial rami of the vagus and a branch to the ramus visceralis. These sympathetic rami consist almost entirely of non-medullated fibres; only very rarely have one or two medullated fibres been seen, in many animals there certainly are none. These medullated fibres are smaller in diameter than most of the fibres in the vagus and they might be either pre-ganglionics or visceral sensory fibres. It has been impossible to decide this point, which is of considerable theoretical importance in connexion with the question of dorsal and ventral root outflows (see p. 528). Since these fibres are so few in number they can have relatively little functional importance and for the present they are tentatively regarded as of sensory nature. In the diagram (Text-fig. 12) they are represented by dotted lines. Experiments in which the vagus was cut inside the skull would show whether there are pre-ganglionic fibres passing to the sympathetic by this route.

The chain in front of the vagal sympathetic ganglion is narrower than that behind on account of the fibres which have ended in the ganglia. It runs forwards close to the lateral wall of the skull and mesial to the anterior cardinal vein towards the glossopharyngeal nerve which it crosses ventrally, mesial to the ganglion. Sometimes there is also a thinner dorsal loop round the nerve. The glossopharyngeal sympathetic ganglion is a small swelling on the chain behind the ninth nerve to which it is joined by a ramus communicans composed almost entirely of non-medullated fibres. As in the case of the vagus, this ramus sometimes also contains a few medullated fibres.

In front of the glossopharyngéus the chain sometimes bears

a small ganglion and then curves dorsally along the outer wall of the skull to the facialis. Before reaching the latter it becomes enclosed in a wing of the prootic bone. Behind the hyomandibular nerve there is a small sympathetic ganglion from which a ramus passes to the hyomandibular. This ramus consists mostly of non-medullated fibres but may contain also some medullated fibres. The chain loops dorsally and ventrally round the hyomandibular trunk and runs forwards into the trigeminal sympathetic ganglion.

12. Trigeminal Sympathetic Ganglion.

This is a polygonal structure, about 1-2 mm. long, flattened against the ventral surface of the Gasserian ganglion. It varies much in shape in different individuals and especially in its relations to the palatine nerve, which may pass through the middle of the ganglion (Text-fig. 7) or on one side of it (Text-fig. 9).

The fibres running forward into the ganglion from the chain separate out and lose themselves among the cells; none can be seen to run right through the ganglion, but it is possible that a few do so.

Many nerves issue from this ganglion all round its circumference. The rami communicantes to the trigeminal nerve are composed mostly of non-medullated fibres, but there are usually a few medullated fibres as well (see p. 513). A large nerve of non-medullated fibres is given off backwards to the arteries of the head (*z*, Text-fig. 9). There are several fine nerves to the wall of the orbit (*n. orb.*) and these are peculiar in being made up of medullated fibres, although they are probably post-ganglionic.

The relations of the ciliary roots are very variable since the junctions and divisions may take place at different distances along the course of the nerves. It is interesting to note that the arrangement is always the same on the two sides of any one animal. The conditions seen in Text-fig. 9 will first be described (see also diagram, Text-fig. 8). Fibres from the trigeminal sympathetic ganglion run in both the long and the short ciliary nerves. In the figure one radix sympathica can be seen running

TEXT-FIG. 8.

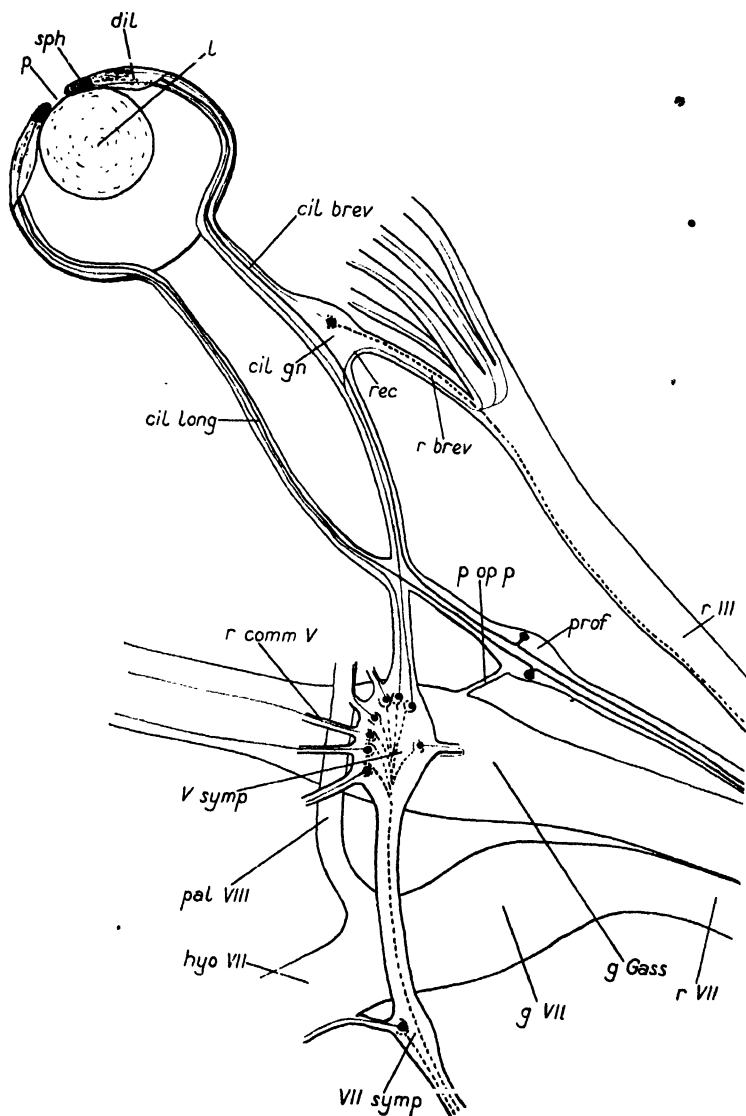
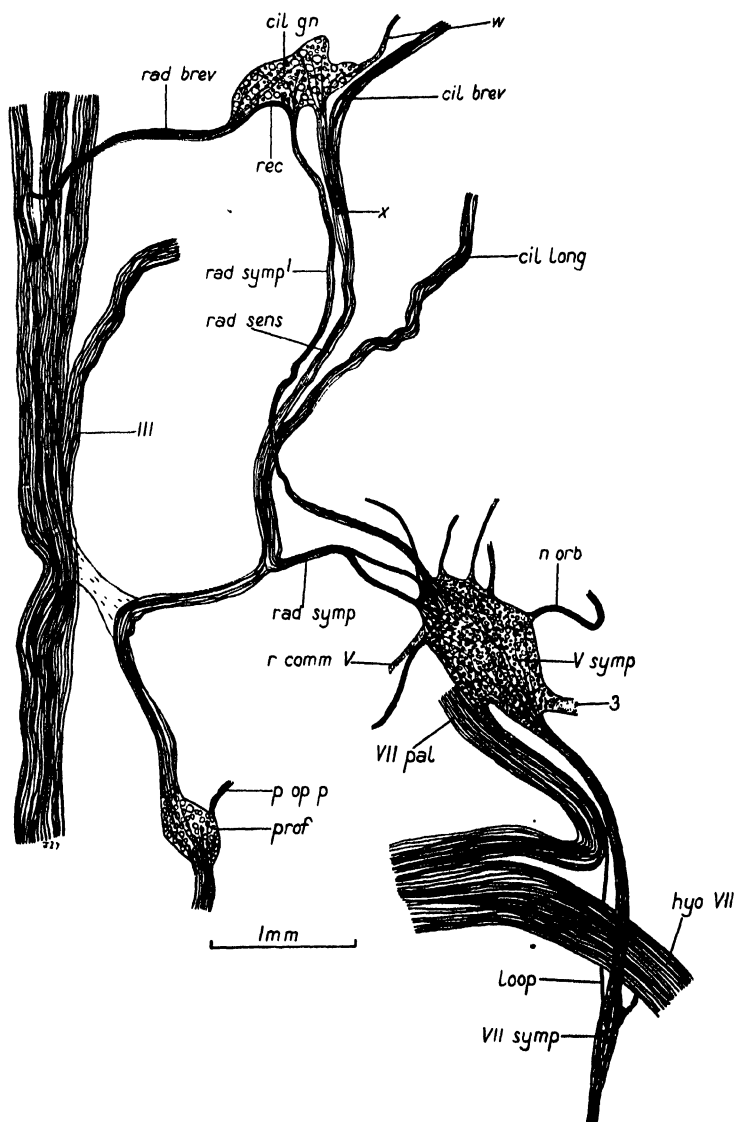


Diagram of the relations of the sympathetic system with the anterior cranial nerves. Sensory fibres: solid black lines; pre-ganglionic fibres: broken lines; post-ganglionic fibres: red lines.

TEXT-FIG. 9.



Left trigeminal sympathetic and ciliary ganglia. Ventral view. Cam. luc.

to the ciliary ganglion and two to the long ciliary nerve. These sympathetic branches to the ciliary nerves consist mainly of medullated fibres, although anatomical (p. 514) and experimental evidence (Young, 1931) shows them to be post-ganglionic.

The sympathetic fibres for the long ciliary nerves join the profundus in the palatine canal. Soon after the junction a part of the profundus splits off as the sensory root to the ciliary ganglion. The long ciliary nerve itself runs dorsally alongside the superior rectus muscle to the back of the eyeball.

In other animals the profundus splits into two before it is joined by the sympathetic roots and in still other cases the profundus and sympathetic roots all meet at one point and run together for some distance before dividing. Often there are a few sympathetic cells at the point of junction of the radix sympathica and the profundus. This corresponds with the morphological position of the other cranial sympathetic ganglia, namely, at the point of junction of the dorsal root with the sympathetic chain. This collection of cells may therefore be considered as representing part of the sympathetic ganglion of the first head segment; the other part being the ciliary ganglion, placed at the junction of the ventral root (oculomotor) with the sympathetic chain (radix sympathica).

13. Ciliary Ganglion.

This is a tiny structure, less than a millimetre long, lying near the oculomotor and just ventral to the optic nerve. It is best found by opening the orbit from the ventral side and cutting the inferior rectus muscle and the nerve to the anterior rectus. It receives rami from the oculomotor (radix brevis), trigeminal sympathetic ganglion (radix sympathica), and from the profundus. In many individuals the sympathetic and sensory roots are bound up together to give a single radix longa, it is exceptional to find them separate as in Text-fig. 9.

Some of the fibres of the radix sympathica seem to end in the ciliary ganglion; the nature of these fibres is uncertain. Possibly they are pre-ganglionic fibres which have run right through the trigeminal sympathetic ganglion. Other fibres of the sympathetic root run through the ciliary ganglion into the

short ciliary nerves, and yet others (*rec.*, Text-figs. 9 and 10) turn back into the radix brevis with which they run to the oculomotor. This is a most peculiar course, but it is certain that it has been correctly interpreted since in animals in which the oculomotor has been cut for some time (see Young, 1931)

TEXT-FIG. 10.

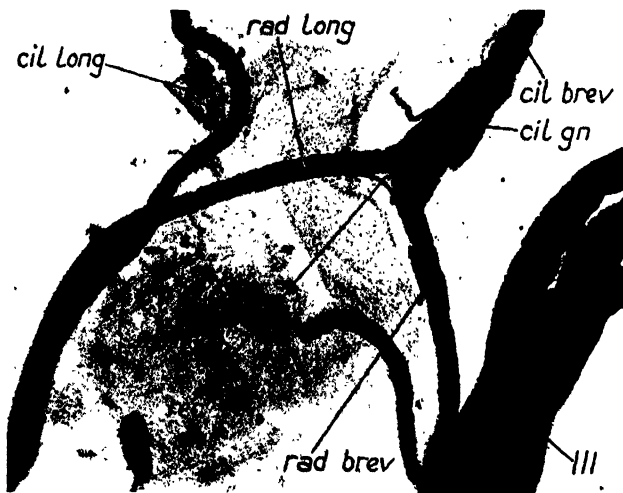


Photo-micrograph of the ciliary ganglion. Magnified 36 \times .

these fibres show no changes, although the centrifugal fibres in the radix brevis are degenerating (Text-fig. 11). When they reach the oculomotor, the fibres of this tract divide into three nerves, two running peripherally and one accompanying the fibres of the radix brevis towards the brain (*rad. brev.*¹, Text-fig. 11). That these fibres are sympathetic and not sensory is shown by the arrangement in Text-fig. 9 where they can be traced to their origin from the trigeminal sympathetic ganglion. Presumably these fibres run with the branches of the oculomotor nerves to the eye-muscles. Probably they are the fibres responsible for the withdrawal of the eye-ball which is seen on stimulation of the sympathetic chain (see Young, 1931). In mammals it is known that sympathetic fibres run to the eye-muscle nerves.

The centrifugal fibres of the radix brevis are fine and medullated like those of the white rami of the trunk. They all end in the ciliary ganglion as is shown by the fact that after section of the oculomotor nerve, degenerating fibres are found in the radix brevis but not in the short ciliary nerves (Text-fig. 11). The post-ganglionic fibres of the short ciliary nerve are also medullated. This nerve runs between the ophthalmic artery and vein, close to the ventral side of the optic nerve, near to which it enters the eye-ball.

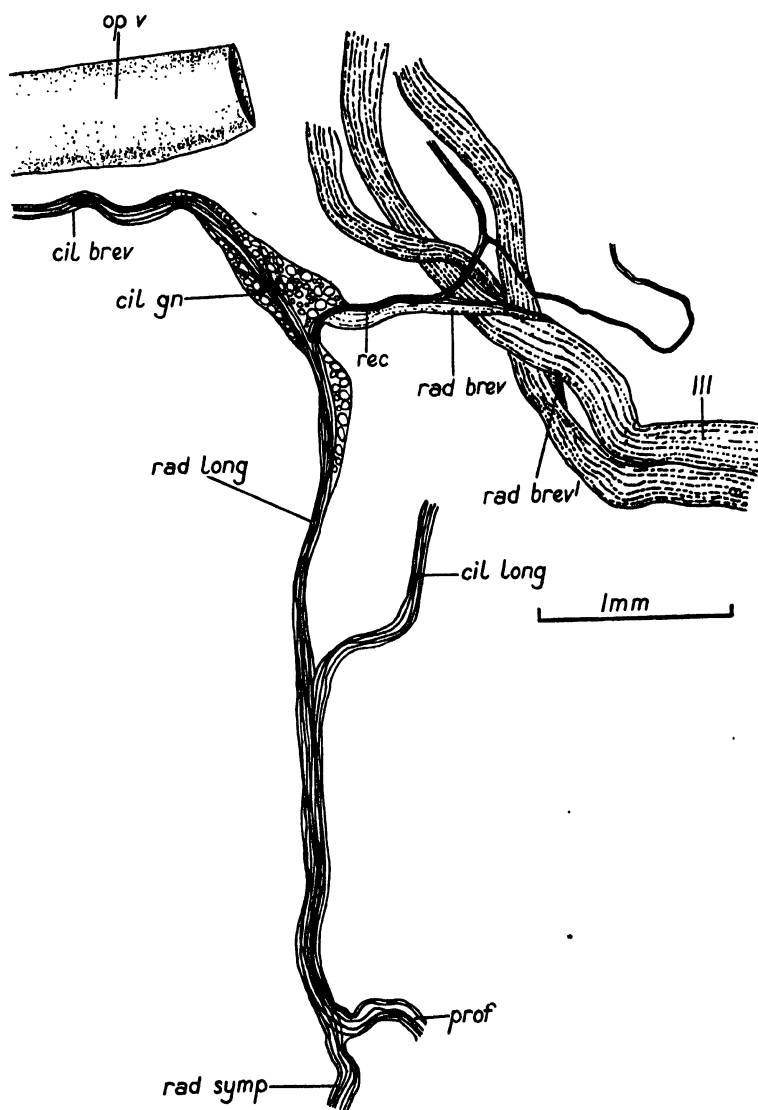
The ciliary ganglion is placed sometimes at the point of junction of the radix brevis, radix longa, and short ciliary nerve (as in Text-fig. 11), and sometimes on the radix brevis itself (Text-fig. 9). In the latter case the post-ganglionic fibres run backwards for a short distance along the radix longa and then forwards at the point *x* in the short ciliary nerve.

The ciliary ganglion of *Uranoscopus* thus resembles that of Tetrapods very closely in that it receives nerves from three functionally distinct sources. In Selachians (Norris and Hughes, 1920) there are sensory and oculomotor roots but no sympathetic root. It would be very interesting to investigate the pupillary mechanism of Selachians to find out how this is affected by the absence of a cranial sympathetic.

Since, in Teleosts, there is a sympathetic ganglion in connexion with the trigeminal and also a connexion between this ganglion and the ciliary ganglion (radix sympathica) it is reasonable to consider the latter as a further extension forward of the sympathetic chain. The ciliary ganglion would thus be the first sympathetic ganglion of the body. Its morphological, embryological, and histological relations are certainly those of a sympathetic ganglion, and it is interesting to find it thus linked up with the rest of the sympathetic ganglia. It is pointed out on p. 530 that the 'antagonistic' action of the sympathetic and oculomotor outflows is no more reason for regarding them as members of different functional systems than is the 'antagonistic' action of the nerves to the flexors and extensors of the limbs.

However, embryological evidence (see p. 529) seems to show that the ciliary ganglion develops independently of the rest of

TEXT-FIG. 11.



Right ciliary ganglion. The oculomotor nerve had been cut 18 days previously. Cam. luc.

the cranial sympathetic ganglia and that the linking up of the ciliary ganglion with the rest of the sympathetic chain is secondary and not primitive (see p. 531).

14. Results from Serial Sections.

The modified Weigert method used is described on p. 496. The preparations proved to be very satisfactory for showing the composition of the nerves, size of fibres, &c., but not so good as the osmic acid preparations for tracing the courses of the fibres through the ganglia.

The sympathetic chain between the glossopharyngeal and facial nerves consists of about equal parts of non-medullated and fine medullated fibres. The latter are about $4-8\mu$ in diameter (the sensory fibres of the facial nerve vary from $5-10\mu$, the motor fibres of the oculomotor are 12μ or more in diameter).

The ramus between the hyomandibular nerve and its sympathetic ganglion consists mostly of non-medullated fibres, together with a few medullated fibres (see p. 514). The rami between the trigeminal and the sympathetic consist only of non-medullated fibres in the preparations examined (see, however, p. 514). There are two large rami of non-medullated fibres which run forward with the palatine branch of the facial.

The sympathetic rami to the ciliary nerves consist of non-medullated and medullated (4μ) fibres in about equal proportions. All of the non-medullated fibres run to the ciliary ganglion. There are no non-medullated fibres in the short ciliary nerve, so those in the radix sympathica must either end in the ciliary ganglion, or acquire sheaths, or run out in the various fine nerves which leave the ciliary ganglion for the blood-vessels of the orbit.

The fibres of the profundus nerve are all medullated but vary in size from 7μ to 1.5μ . The very fine fibres are all in one part of the nerve and they all run in the radix longa and short ciliary nerve.

To summarize: the long ciliary nerve contains only medullated fibres, derived from the sympathetic and profundus; the radix longa contains medullated and non-medullated sympathetic fibres and fine and very fine profundus fibres; the short

ciliary nerve contains the same profundus fibres as the long root and also medullated fibres from the sympathetic and from the ciliary ganglion.

15. Summary of Cranial Sympathetic System.

In *Uranoscopus* there is a sympathetic ganglion in connexion with the oculomotor nerve (ciliary ganglion) and with each of the dorsal cranial nerves. These ganglia are joined together by a chain and to the cranial nerves by means of rami communicantes. Except in the case of the oculomotor (*radix brevis*) these rami consist almost entirely of non-medullated fibres; there are, however, in every segment one or two medullated fibres, but there is no evidence whether these are sensory or pre-ganglionic visceromotor fibres.

The main supply of pre-ganglionic fibres for the sympathetic ganglia of the head runs out in the rami communicantes of the third and fourth spinal segments. These fibres run forward as a large bundle from which fibres branch off to end in the first two spinal, and cranial sympathetic ganglia. No fibres can be seen to run right through the trigeminal sympathetic ganglion into the ciliary roots, but it is possible that some do so, since certainly some of the fibres of the *radix sympathica* end in the ciliary ganglion.

The fibres from the trigeminal sympathetic ganglion which run to the intrinsic eye-muscles are medullated, although it is practically certain that they are post-ganglionic; they run in both long and short ciliary nerves. There is a bundle of fine medullated fibres, arising from the trigeminal sympathetic ganglion, which passes via the *radix longa*, ciliary ganglion, and *radix brevis* to the oculomotor. These fibres correspond, morphologically, to the recurrent (grey) rami communicantes of the trunk. The fibres probably end in the extrinsic eye-muscles.

The *radix brevis* also contains medullated, pre-ganglionic fibres which end in the ciliary ganglion.

COMPARISON OF THE AUTONOMIC NERVOUS SYSTEM OF TELEOSTS
WITH THAT OF OTHER CLASSES.

A. Trunk Region.

The plan of the sympathetic in the spinal region of Teleosts agrees closely with that found in other classes. In that each ganglion is connected with the spinal nerve by white and grey rami, the Teleost agrees with the Tetrapod, but differs from the Selachian, in which (as will be shown in a future paper) there are no recurrent (grey) rami to the spinal nerves.

The sympathetic chain seems to resemble that of Tetrapods in consisting mainly of pre-ganglionic fibres with an admixture of some post-ganglionics. Here, again, the Teleost differs from the Selachian in which, although there is almost always a connexion between the sympathetic ganglia of adjoining segments, yet this chain never contains medullated (pre-ganglionic) fibres.

The innervation of the viscera of Teleosts differs somewhat from that of Tetrapods (see p. 509). All the fibres for the gut run backwards in the vagus (which contains also a large number of fibres from the sympathetic) and splanchnic nerves. There is no separate innervation of the hind part of the gut and nothing has been found corresponding to the sacral parasympathetic outflow which is present in all Tetrapods (for Amphibia see Langley and Orbeli, 1911 and 1912). The condition in Selachians is not known with certainty. There are apparently nerves from the sympathetic ganglia to the hind part of the gut, and Bottazzi (1902) reported that stimulating the spinal cord in one limited region caused movements of the intestine. However, Müller and Liljestrand (1918) were unable to observe any movements of the gut on stimulation of the ventral roots in this region.

The mesonephros and its derivatives (kidneys, bladder) and the gonads, receive their nerves only from the sympathetic system; there is apparently no double innervation of these organs, such as is found in Tetrapods.

B. Cranial Region.

The Teleosts are unique among vertebrates in that the ganglionated sympathetic chain extends forwards into the head,

TEXT-FIG. 12.

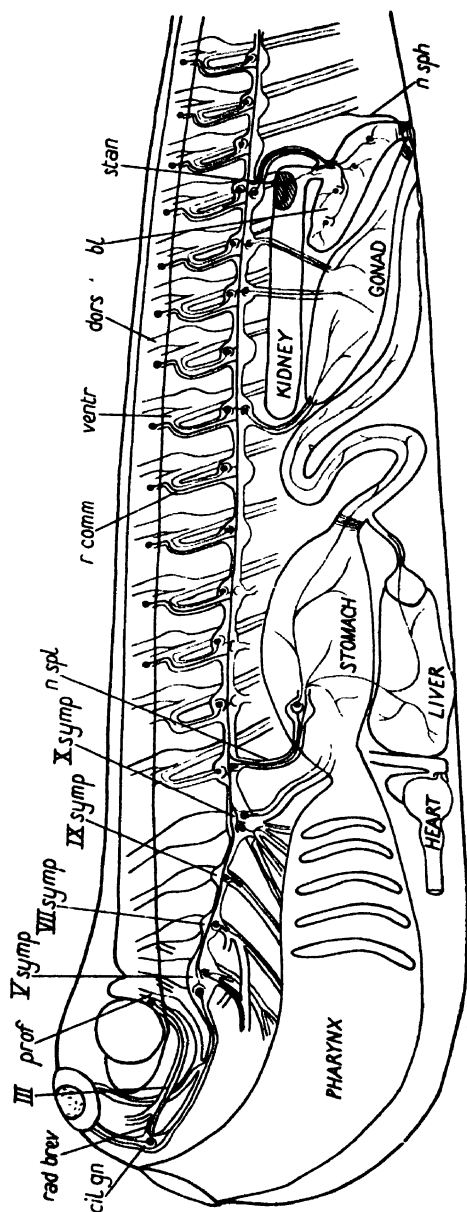


Diagram of the autonomic nervous-system of *Uranoscopus* in lateral view. Pre-ganglionic fibres: solid black lines; post-ganglionic fibres: red lines.

bearing ganglia in connexion with the cranial nerves. The case of the ciliary ganglion has already been considered (p. 519). In many groups it has been shown that it resembles a typical sympathetic ganglion in development (partly from the trigeminal ganglion and partly from cells which wander out along the oculomotor), in morphological position (at the junction of a ramus from a ventral root with the sympathetic chain) and histologically. Moreover, the fibres which run back in the *radix brevis* to the oculomotor nerve correspond to a recurrent (grey) ramus communicans. There is, in Teleosts, another collection of cells which, with the ciliary ganglion, must be considered as representing the sympathetic ganglion of the first head somite, namely, the cells found at the point of junction of the profundus (dorsal root) with the sympathetic chain (*radix sympathica ad ganglion ciliare*).

The chain of sympathetic ganglia in connexion with the dorsal cranial nerves of Teleosts has no homologue in any other vertebrates, although there are often connexions between the sympathetic and cranial nerves. In Selachians there is sometimes a thin ramus connecting the first sympathetic ganglion with the vagus but it is as often absent and its nature has not yet been determined. The conditions in 'Ganoids' are not fully known. Chevrel (1894) found that in *Acipenser* there are connexions between the front end of the sympathetic chain and the vagus and glossopharyngeus, but that there are no sympathetic ganglia in the head. Allis (1897) figures sympathetic chains extending into the head of *Amia* but gives no further description. Norris (1925) states that the sympathetic is well-developed in *Holostei* but gives no account of it. In the *Dipnoi*, according to Jenkin (1928), there is not even a connexion of the sympathetic with the vagus.

In Tetrapods there are usually rami from the superior cervical ganglion to the vagus, hypoglossus, and glossopharyngeus, and indirectly, via the carotid plexus, to the facialis, trigeminus, and eye-muscle nerves. The nature of the vagus-sympathetic connexion has been investigated in *Amphibia* by Langley and Orbeli (1911 and 1912), and in mammals by numerous authors (van der Broeke, 1908; Billingsley and Ranson, 1918; Langley,

1921; Iwama, 1928; Duncan, 1927). The ramus contains medullated as well as non-medullated fibres, the former being usually interpreted as post-ganglionic or sensory and not as pre-ganglionics. Iwama claims that after cutting the vagus in the cervical region in cats, degenerating medullated fibres are found in the sympathetic both in front of and behind the point of junction of the vagus and sympathetic, but he brings no evidence as to their nature, probably they are sensory.

In *Uranoscopus*, as has been shown above, the rami communicantes between the sympathetic and the cranial nerves consist chiefly of non-medullated fibres with the admixture of a few medullated fibres. The non-medullated fibres are presumably post-ganglionics which are distributed to their end organs with the cranial nerves; as to the nature of the medullated fibres there is unfortunately no evidence, they might be pre-ganglionic visceral motor fibres or viscerosensory fibres. It would be of great interest to decide this question in view of its importance in considering the homologies of these ganglia (see p. 528).

The question arises whether the cranial sympathetic ganglia of Teleosts can be considered homologous with the autonomic ganglia found in the head of other classes of vertebrates in connexion with the dorsal root nerves. The chief of these, in mammals, are the sphenopalatine, otic, and submaxillary ganglia which receive pre-ganglionic fibres via the facialis and glossopharyngeus (dorsal roots) and histologically resemble sympathetic ganglia. These ganglia may also receive branches from the superior cervical sympathetic ganglion (see, for instance, Goodrich, 1930, p. 463, for an illustration of a large sympathetic-sphenopalatine connexion). The nature of these rami is not known, but it is usually assumed that they consist simply of post-ganglionic sympathetic fibres which pass through the ganglia on their way to the salivary glands.

Autonomic ganglia in similar positions have been found in birds and reptiles (see Willard, 1915). In Amphibia, Coghill (1902), Norris (1908), and Norris and Hughes (1918) report the presence of a small ganglion on the ramus palatinus facialis, just where the latter anastomoses with the ramus maxillaris trigeminus. This is exactly the position of the sphenopalatine

ganglion of mammals with which the amphibian ganglion seems to be homologous. No other comparable ganglia have been found in the head of Amphibia. •

No mention of any autonomic ganglia along the course of the cranial nerves of 'Ganoids' is to be found in the works of Allis (1897) or Norris (1925).

In Selachians, however, Norris and Hughes (1920) report the presence of what they take to be 'sympathetic' ganglia on the post-trematic branches of the facial, glossopharyngeal, and vagal nerves; there is apparently no one of these which corresponds to the sphenopalatine ganglion of Tetrapods, but the ganglia on the facial and glossopharyngeal might reasonably be homologized with the submaxillary and otic ganglia respectively, especially as they receive their connexions (pre-ganglionic?) via the dorsal roots. Hoffmann (1900) believed that the sympathetic ganglia of the head of Selachians were included in the cranial nerves; he points out that fibres from these nerves innervate the blood-vessels of the head and the thymus. It has often been suggested (L. R. Müller, 1910; Kidd, 1914; E. Müller, 1920; Hess and Pollack, 1926) that the vagus ganglion of Selachians (and other groups) contains visceromotor cells. This would be very interesting if proven, but the evidence is at present conflicting.

Our knowledge of the autonomic nervous system of Cyclostomes is too fragmentary to allow of comparison with that of other classes. Johnston (1905) reported the presence of a ganglionated chain in the branchial region of *Ammocoetes*, but he was unable to confirm Julin's (1887) finding of a series of ganglia in the trunk. Tretjakoff (1927) confirms Johnston's statements about the branchial ganglia but does not describe them fully or critically. He also reports the presence of sympathetic cells in connexion with the olfactory and optic nerves.

In Teleosts I can find no mention of any ganglia along the peripheral courses of the cranial nerves, but they have never been searched for systematically and may have been overlooked. It has to be considered whether the segmental cranial sympathetic ganglia are homologous with the autonomic ganglia in the head of other animals. As regards their position, they

lie much nearer to the cranial roots than do any of the ganglia of Tetrapods or Selachians mentioned above. The trigeminal sympathetic ganglion is in approximately the same morphological position as the sphenopalatine of Tetrapods, namely, at the crossing of the palatine and trigeminal nerves. The sub-maxillary and otic ganglia must have shifted far along the corresponding cranial nerves if they are homologous with the facial and glossopharyngeal sympathetic ganglia of Teleosts.

There are, however, more serious objections to homologizing any of these cranial sympathetic ganglia with the ganglia in the head of Tetrapods. Whereas the latter receive their pre-ganglionic fibres via the corresponding dorsal root nerves, in the sympathetic of Teleosts all the pre-ganglionic fibres run out in the ventral roots of the more posterior spinal segments and thence forwards into the head (excepting always the possibility that some of the few medullated fibres in the rami communicantes of these cranial ganglia may be pre-ganglionic). Apart altogether from the question whether any great morphological significance is to be attached to the passage of visceromotor fibres by way of the dorsal or ventral roots (see p. 530) it must be admitted that the difference in the course of the fibres is such as to make it improbable that these ganglia of Teleosts are homologous with any autonomic ganglia which receive their pre-ganglionic fibres via the dorsal cranial nerves.

It would appear, then, that the more peripheral autonomic ganglia of the head are a special acquirement, presumably in connexion with the elaboration of the salivary glands, and this is confirmed by the fact that they are poorly developed in Amphibia and less developed in reptiles than in mammals (Willard, 1915). Possibly there were, in the ancestors of the Tetrapods, collections of ganglion cells on the post-trematic branches, such as are found to-day in Selachians, or possibly the latter cells are of quite different nature; fuller study of them is desirable before making further generalizations.

As regards the true sympathetic system it seems that in Tetrapods post-ganglionic rami pass forwards to the cranial nerves from a ganglion lying behind the vagus, whereas in Teleosts pre-ganglionic fibres run forwards into the head to

connect with sympathetic ganglia in each cranial segment. Can the latter be considered as a more primitive condition from which the Tetrapod arrangement is derived by coalescence of the ganglia? In any primitive metameric arrangement of ganglia it would be expected that there would be an outflow of connector elements in every segment; in Teleosts there are no such segmental outflows in the head. It might be that the present arrangement is the result of the specialization of the ventral roots (by which the outflow must take place) in the head, so that the sympathetic fibres have perforce to come out more posteriorly, but this is hardly an adequate explanation. The suspicion arises that these segmental cranial sympathetic ganglia represent a secondary and not a primitive arrangement.

This suspicion is strengthened by a consideration of the embryological data. Schwartz (1919) states that the sympathetic elements grow forward into the head of the trout so that, even in 2.5 cm. larvae, the chain reaches only to the front of the glossopharyngeus and in 3 cm. larvae to the facialis. He therefore considers that the whole cranial sympathetic is formed by fibres and cells which have grown forward from the anterior spinal nerves. This conflicts with the rather meagre statements of His jr. (1892) to the effect that the cranial sympathetic system is already formed in 2 cm. larvae and that the ganglia develop from the cranial nerves. This question certainly needs answering since it contains the key to the problem. If the cranial sympathetic ganglia of the Teleosts are primarily segmented they would be expected to develop from the cranial ganglia as do the sympathetic ganglia of the trunk (at least in part; for a summary of the literature on this problem see Campenhout, 1930), and as does the ciliary ganglion (which in the trout, according to Schwartz, develops earlier and independently of the rest of the sympathetic, only becoming joined up later). The sphenopalatine, submaxillary, and otic ganglia of mammals develop from cells which pass out via the cranial ganglia.

Furthermore, it is not in all Teleostei that the cranial sympathetic ganglia are strictly segmental; Chevrel (1887) found that in the Apoda (*Anguilla*, &c.) the most anterior sympathetic ganglion is placed on the glossopharyngeus and that

from this ganglion branches pass to the other cranial nerves. These are 'physostomatous' fishes which show many primitive characters. This lends further weight to the hypothesis that the cranial sympathetic has been developed within the teleost series and that it is not to be regarded as a primitive character. This was the conclusion reached by Chevrel (1894). He points to the gradual increase in the development of the cranial sympathetic as we pass from the Selachians, through the 'Ganoids' and the Apoda to the rest of the Teleosts. It is perhaps safer, for the present, to consider the Selachians apart and not as primitive forms, but certainly the presence of a chain of cranial sympathetic ganglia only in the more highly specialized modern fish and not in the more primitive forms, taken together with the embryological and anatomical evidence, points to the conclusion that the present arrangement is of fairly recent origin.

MORPHOLOGY OF THE AUTONOMIC NERVOUS SYSTEM.

As has been pointed out by Goodrich (1927 and 1930) the autonomic nervous system consists of two morphologically distinct sets of fibres, one leaving in the dorsal and one in the ventral roots. Moreover, the ventral root outflow corresponds in general to the 'sympathetic' system of the physiologist and the dorsal root outflow to the 'parasympathetic', which, however, also comprises fibres leaving in the oculomotor nerve (a ventral root) and in the ventral sacral roots. These prosomatic and sacral outflows differ, however, in many ways from the rest of the parasympathetic and are in fact classed with the latter chiefly on account of their 'antagonism' to the sympathetic. Schilf (1927) has pointed out that the effect of the oculomotor and 'sympathetic' outflows on the pupil is better described as synergic than antagonistic, and that there is no more reason for referring these nerves to different functional systems than there is in the case of the nerves to the flexor and extensor muscles of a limb, all of which nerves can be classed together as somatic motor.

It is, then, conceivable that the dorsal and ventral root outflows of mammals are the remains of a previous metameric arrangement in which there was a complete double series of

viscero-motor fibres, leaving in the dorsal and ventral roots of every segment throughout the whole body. We have to inquire whether there is any evidence from the fish that this was the case.

The extension of the sympathetic chain into the head of Teleosts seems at first sight to constitute such a primitive arrangement. There is, in fact, a complete series of true sympathetic ganglia (connected, that is to say, with the ventral roots) extending from the ciliary ganglion to the tip of the tail. Closer examination has revealed, however, that there is not actually an outflow of pre-ganglionic fibres in the ventral roots of every head segment, and that the whole sympathetic system of the head (with the exception of the ciliary ganglion) is probably a secondary development and does not represent a primitive segmental arrangement. These conditions do not, in fact, lend support to the view that there was ever a complete series of ganglia connected with the ventral roots in every segment, but rather suggest that the ventral root system arose rather late in phylogeny, after the specialization of the cranial region, and that it only secondarily grew forwards into the head. This is supported by the fact that a ventral root system occurs only in the trunk region of Selachians and Acipenser and does not extend into the head in them.

The primitive condition, then, may have resembled that found in *Amphioxus* (Hatschek, 1892; Franz, 1927) where the ventral roots innervate only the myotomes and all viscero-motor fibres leave by the dorsal roots. The motor cells may then have migrated out along the dorsal roots and become aggregated into separate sympathetic ganglia which secondarily became connected with the ventral roots. This is certainly the course taken by the sympathetic cells in ontogeny (although probably some cells also migrate out along the ventral roots). Further study of the autonomic system of fish and especially of cyclostomes is needed to settle these questions.

SUMMARY.

1. The sympathetic ganglia of *Uranoscopus* resemble those of mammals in that they are connected with the spinal

nerves by white rami consisting of medullated (pre-ganglionic) fibres, and grey rami of non-medullated (post-ganglionic) fibres.

2. The sympathetic ganglia are connected together by a chain in which the medullated fibres may run forwards or backwards and may also cross in transverse commissures to the opposite chain.

3. No sympathetic nerves to the heart were found. The cardiac nerves spring from the vagus and consist of medullated fibres.

4. The gonads and the mesonephros and its derivatives (kidneys, urinary bladder) receive nerves only from the sympathetic system, with the exception of the sphincters round the external openings of the gonads and bladder which are innervated by branches of the spinal nerves.

5. There is, on each side, only a single anterior splanchnic nerve from the sympathetic system to the gut; there is therefore nothing corresponding to the pelvic nerves (sacral parasympathetic outflow) of Tetrapods.

6. The sympathetic chain extends into the head and bears sympathetic ganglia in connexion with the vagus, glossopharyngeus, facialis, and trigeminus nerves. The rami communicantes between these sympathetic ganglia and the cranial nerves consist almost entirely of non-medullated (post-ganglionic) fibres; the pre-ganglionic fibres for these ganglia run out in the white rami of the third and fourth spinal nerves and thence forwards in the sympathetic chain.

7. The ciliary ganglion resembles that of mammals, receiving fibres from the trigeminal sympathetic ganglion (radix sympathica), profundus ganglion (radix sensoria) and oculomotor (radix brevis). The sympathetic fibres in the long and short ciliary nerves (though post-ganglionic) are medullated. There is a bundle of fibres running from the trigeminal sympathetic ganglion via the radix longa and radix brevis to the oculomotor nerve.

8. There is a separate profundus ganglion in *Uranoscopus* from which a small nerve passes to join the trigeminus (portio ophthalmica profundi?) while larger branches run to the long and short ciliary nerves.

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Micronuclear Variation in *Paramecium bursaria*.

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With 1 Text-figure.

THE dimorphic condition of the nuclear apparatus of the Infusoria is one of the most interesting and characteristic specializations exhibited by the group, apparently representing a segregation of 'somatic' and 'germinal' elements into distinct macronuclei and micronuclei. Accordingly these bodies have been extensively studied during the various life-processes of the Infusoria. Furthermore, several investigators have described races of well-known species of Infusoria in which no morphologically differentiated micronucleus is present, and it is highly significant that such amiconucleate animals when carefully cultured are viable but unable to consummate conjugation. They are amiconucleate both structurally and functionally (1).

In view of these facts and the importance of the structure and number of the micronuclei in determining the relationships of the several species of the genus *Paramecium* (2), the following account records variations in the micronuclear number, &c., in a pedigree race of *Paramecium bursaria*—variations ranging from four micronuclei to the absence of a micronucleus in genetically related animals.

During July 1924, specimens of *P. bursaria* collected from a small freshwater pond at Woods Hole, Massachusetts, revealed the presence of two micronuclei of the 'caudatum type', instead of the single micronucleus of the same type characteristic of *P. bursaria*. Accordingly a pedigree race of bi-miconucleate animals was started on July 26, 1924, and since that time to the present (July 1931) it has been cultured either in small mass cultures or in daily isolation cultures at

the Osborn Zoological Laboratory of Yale University. The culture medium used has been hay infusion. At various periods during these seven years cytological studies of the nuclear apparatus of the animals has been made. Derivatives of this culture were employed by Dr. R. C. Parker in his investigations on symbiosis (3).

OBSERVATIONS.

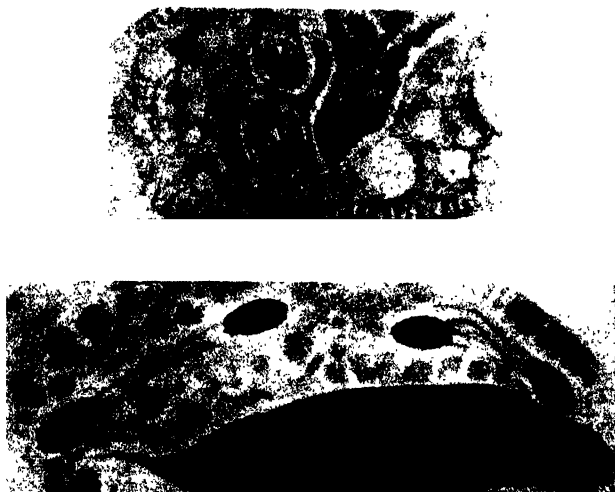
Pedigree daily isolation slide cultures were carried first from July 26 to August 30, 1924. During this period two micronuclei were consistently present, each exhibiting all the characteristics of the single micronucleus typical of *P. bursaria*. The condition is well illustrated by Text-fig. 1. The results obtained up to this point apparently would justify the establishment of a new species, characterized by two micronuclei of the 'caudatum type'—a species that, *a priori*, might be expected to exist from the intrageneric relationships of the known species of *Paramecium*. But nearly fifty generations in pedigree culture proved to be insufficient for a crucial criterion—although a far more rigid test than most zoological species have been, or can be, subjected to by taxonomists.

On October 1, 1924, several daily isolation slide cultures were started from a small mass culture derived from the first isolation culture, and in one of these, on October 5, the animals proved to have but a single micronucleus. Accordingly during the next few months a large number of similar isolation cultures were started from the same small mass culture and were carried for periods of different lengths. Some of these comprised animals with two, and some with one micronucleus. The unimicronucleate lines quite consistently bred true, but gradually all of the bimicronucleate lines became unimicronucleate. No evidence of endomixis was observed.

The process of transformation from the bimicronucleate to the consistently unimicronucleate condition was fairly gradual and seemed to be the result of a general instability of the micronuclear number that culminated during March and April 1925. During this period variation in micronuclear number ranged from one to as high as four in several cases, but finally

all the lines of the culture became stabilized with one micronucleus characteristic of *P. bursaria* during June 1925—nearly one year after the bimicronucleate ancestor was isolated in pedigree culture.

The now typical culture of *P. bursaria* was carried in mass



B

TEXT-FIG. 1.

P. bursaria showing bimicronucleate condition. A. Two micronuclei in an animal from a pedigree culture at the 22nd generation, August 13, 1924. B. Division of the two micronuclei in an animal from the same culture, July 27, 1924. Total preparations: Delafield's haematoxylin; Zeiss apoch. oil imm. 1·30, comp. oc. 12.

cultures in small beakers without especial attention to its nuclear condition for more than four years. Then, in January 1930, an intensive cytological investigation of the race was resumed by the study of animals in daily isolation and small mass cultures. This study, carried on with the assistance of Miss Rosaltha Sanders of the Osborn Laboratory, and supported, in part, by a grant from the Bache Fund of the National Academy of Sciences, was undertaken in order to determine the micronuclear condition of the race after the lapse of years

and also to renew the search for endomixis, since this process was not observed in the previous intensive study, made before Erdmann's observation of endomixis in Pringsheim's culture of *P. bursaria* (4).

The immediate source from which the pedigree isolation cultures were started was two mass cultures in flasks. These mass cultures, designated G and H respectively, were, of course, genetically related. H had been seeded from G during September 1929. Examination of the animals showed that all those in culture G possessed one micronucleus, quite characteristic in general form but highly refractory to 'chromatin stains'. Indeed, only in very rare instances was 'chromatin' demonstrable, the micronucleus appearing as an achromatic 'ghost'. This condition of the micronucleus has persisted in culture G and its derivatives to the present time (July 1931). Furthermore, no evidence of endomixis was found. Since both daily isolation and small mass cultures were studied in 1924, 1925, and again in 1930 and 1931, it seems positive that endomixis does not occur in this race of *P. bursaria* under the culture conditions provided, which have proved to be highly favourable for the multiplication of the animals. Certainly endomixis is not responsible for the micronuclear variations that have been observed.

The similar study that was made on animals from culture H revealed not only further progress in the loss of affinity of the micronuclei for chromatin stains, but also the disappearance of even micronuclear ghosts. Among the large number of animals examined, only two revealed micronuclei, a single micronucleus in each, and these were not animals whose ancestors had shown the absence of micronuclei. Consequently it seems clear that there has been a progressive reduction in the micronuclear apparatus of the race since 1924, culminating in animals of the H culture and its derivatives in which a micronucleus is not demonstrable by the various types of cytological technique entirely adequate to reveal micronuclei earlier in the life-history of the race. The animals have become amiconucleate.

Briefly stated, then, this pedigree race of *P. bursaria* during the seven years under culture has exhibited marked micro-

nuclear instability. Originally bimicronucleate, for a brief period it exhibited from one to four micronuclei, and then settled down, as it were, for several years with the single micronucleus of the 'caudatum type' characteristic of *P. bursaria*. But still later, and up to the present time, derivatives of one mass culture exhibit micronuclear ghosts, while those of another similar culture are amicronucleate. Neither endomixis nor conjugation has ever been observed during the seven-year culture period. The animals are apparently as healthy to-day as in 1924. All the cultures are in a flourishing condition.

DISCUSSION.

Variations in micronuclear number in individuals of certain species of *Paramecium* with the 'aurelia type' of micronucleus are well known. Thus *P. multimicronucleata* has several, usually four micronuclei, *P. polycaryum* has three to eight, usually four micronuclei, and *P. woodruffi* usually has three or four micronuclei. On the other hand, in the species with the 'caudatum type' there is typically but one micronucleus. The only recorded exceptions to the unimicronucleate condition in these species are the temporary bimicronucleate condition of *P. caudatum* cited by Calkins (5), the amicronucleate condition of the same species by Woodruff (6), the bimicronucleate condition in *P. trichium* by Wenrich (7), and finally the micronuclear variations in *P. bursaria* described in the present paper.

The significance of the relative constancy of the micronuclear number in species with the 'caudatum type' is not clear, but the fact remains, and accordingly, in species with this type, the number of micronuclei is usually a criterion of specific value. But the observations just recorded emphasize that extensive numerical variation may occur in species with the 'caudatum type' and therefore long study in pedigree culture is demanded to make certain that a character that seems to be of specific value is actually so.

The problem of the method of origin of the animals of this race with supernumerary micronuclei is interesting. It is possible that the original 'wild' bimicronucleate animal isolated

to start the pedigree culture was a descendant of an irregularly reorganized animal—such irregularities having been recorded by Hamburger in her study of conjugation in *P. bursaria* (8). But with conjugation and endomixis ruled out since the race has been under pedigree culture conditions, apparently precocious micronuclear division or irregular distribution of micronuclei must have occurred during 'somatic' cell division and resulted in the bimicronucleate condition, as recorded by Wenrich in *P. trichium*, by Woodruff in *Oxytricha fallax*, and by Manwell in *Pleurotricha lanceolata* (9).

In support of this method of origin of the micronuclear variations in this race of *P. bursaria*, a specific case may be cited. During March 1925 an animal in a pedigree line at the 23rd generation had one micronucleus, while the two descendants of its sister cell possessed two and four micronuclei respectively. Of the several possible methods of origin of this condition, perhaps the following is most probable. The ancestral animal in the 22nd generation is assumed to have had two micronuclei, both of which divided at the ensuing cell-division, but three of the products passed to one cell and only one to the other cell (the cell killed and studied). If the assumption is correct that the animal which carried on the line possessed three micronuclei, then the observed condition, of two and four micronuclei respectively, in its own offspring may also be accounted for merely by an irregularity in the distribution of one micronucleus of one pair—both of this pair passing to one daughter cell of the 24th generation.

Turning to the method of origin of amicronucleate races, it is possible that in certain cases such animals arise by the transformation of all the 'micronuclei' into macronuclei following endomixis or conjugation as suggested by Woodruff (10). Amicronucleate animals were observed by Prandtl after conjugation in *Didinium nasutum*, but he doubted that animals with a nuclear heritage of this character were viable (11). However, Patten derived a viable amicronucleate race of *Didinium* following conjugation of a typical micronucleate race (12), while Diller noted variations in the micronuclear number after endomixis in *Trichodina* and suggested that

this is the basis of the origin of bimicronucleate and amiconucleate races of Infusoria (13).

On the other hand, such irregularities after conjugation or endomixis are not responsible for the assumption of the amiconucleate condition by the race of *P. bursaria* under consideration, not only because these processes have not been observed, and almost surely do not occur in this race, but also because the loss of the micronucleus has been a gradual process, as evidenced by a decline in affinity for so-called nuclear stains giving rise to micronuclear ghosts before their total disappearance. So it seems certain that the amiconucleate condition in the Infusoria may arise in several ways—by atypical reorganization following conjugation or endomixis, by precocious micronuclear division or irregular distribution of micronuclei during somatic cell-division, and by a gradual 'degeneration' of the micronuclei. The result would be the same in each case—'somatically healthy' animals without micronuclei.

Far more interesting than the exact method of origin of multimicronucleate and amiconucleate animals, in species typically unimicronucleate, is the impressively presented fact that whatever function the micronuclear apparatus plays, the somatic life of the animals is not obviously influenced by profound variations in volume or in distribution of micronuclear material. Moreover, amiconucleate animals do not possess the power to undergo endomixis or conjugation—a significant and crucial proof that the micronucleus is not only morphologically but also actually functionally absent. Indeed, Dawson, in particular, has shown that amiconucleate *Oxytricha* undergo periods in which the pedigree animals attempt conjugation, but it always proves abortive, and instead the animals frequently become cannibalistic (14). The one race of *Paramecium* described by Landis as an 'amiconucleate *P. caudatum*' having the power to conjugate has more recently been shown actually to possess several micronuclei. It is *P. multimicronucleata* and not *P. caudatum* (15). Thus the sexual sterility of amiconucleate animals lends support to the generally accepted analysis of the nuclear apparatus of the Infusoria that identifies the macronucleus and micronucleus as repre-

senting a segregation of 'somatic' and 'generative' elements into discrete bodies during the vegetative life of the animals.

SUMMARY.

Studies on a race of *P. bursaria*, in pedigree culture for more than seven years, lead to the following chief results:

1. Marked variations occurred in the micronuclear number. Originally bimicronucleate, the race later assumed the unimicronucleate condition characteristic of the species, and finally became amicronucleate.

2. No marked variation in the vitality of the race has been observed throughout its life in culture, so that whatever function the micronuclear apparatus plays in the somatic life of the race is not obviously influenced by profound changes in the volume and distribution of the micronuclear material.

3. Evidence of endomixis or conjugation has never been observed in this race.

4. The viability of amicronucleate animals, without the power to undergo endomixis or conjugation, further supports the identification of the macronucleus and micronucleus as a segregation of somatic and generative elements into discrete bodies within the cell.

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The Structure of the Otolith of the Hake.

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With Plates 24 and 25 and 1 Text-figure.

WORK on the life-history of the hake *Merlucius merluccius* Linn. has involved the possibility of assessing the age of the fish by counting the rings which are present in the otolith. The results of this investigation have no place in the present paper, which simply seeks to show to what details in the microscopic structure of the otolith the rings are due.

It is curious that the otolith has attracted so little attention from morphologists and histologists. So far as I know, there has been only one exhaustive description of the microscopic structure of the otolith, that of Immermann on the plaice otolith (1908). Maier (1908) deals less fully with the Cod otolith and Lissner (1925) mentions briefly that his observations on the otolith of the herring confirm Immermann's on the plaice otolith. I cannot agree with some of Immermann's observations, at least as concerns the hake otolith.

The structure has been worked out by a careful examination of fresh entire otoliths, of sections cut and ground thin, and of decalcified entire otoliths and sections. For the finest details of the structure I was obliged to obtain thinner sections than I could myself prepare by grinding, and I am indebted to the Lomax Palaeobotanical Laboratories, of Bolton, for two excellent preparations. The photographs which illustrate this paper are due to the skill of Mr. H. Stokes, of the staff of the Fisheries Laboratory at Lowestoft.

A. THE ENTIRE OTOLITH.

Seen in its natural position in the *Sacculus*, the hake's otolith (*Sagitta*) is an irregularly pear-shaped body, strongly

compressed laterally, tapering to a point posteriorly, and bluntly rounded anteriorly. The lower edge is fairly smooth, and is slightly curved, whereas the upper edge is much more indented, and rises in a fairly straight line from the pointed posterior end to well forward of the centre of the otolith, after which it falls in a smooth curve to the blunt anterior end. In fig. 1, Pl. 24, a group of five hake otoliths may be seen lying on their side, with their inner surfaces upward, photographed against a dark background.

The otolith is somewhat curved, in such a way that the inner surface (that next the brain) is convex, the outer surface concave. The inner surface is relatively smooth, and is scored by the wide and shallow *Sulcus Acusticus*. The outer surface is very rugose.

A number of grooves, concentric and parallel to the margin of the otolith, may usually be seen on the smooth inner surface of the otolith, and are clearly marked in wax impressions of this surface. They are usually not visible on the outer surface.

The hake's otolith is a compound structure, built up of a large number of lobes arranged in one plane, radiating from the middle line, towards the periphery, in such a way that their axes are normal to the margin of the otolith. The lobes may be seen very clearly in fig. 1, Pl. 24; they are fused to one another laterally except at their distal ends, and are broader in the upper half of the otolith than in the lower half. Growth in length and depth of the otolith is accomplished by a branching of the lobes, several of which may be seen dividing, in the otoliths in fig. 1, Pl. 24, and fig. 5, Pl. 25.

A fresh hake otolith, owing to its flattened shape, is very fairly translucent, and, when held against a source of light, is seen to have a discontinuous structure, and to be divided into broad, less translucent zones, separated by narrow, more translucent rings, which are strictly parallel to the margin of the otolith. When placed against a dark background, these more translucent rings appear black, the less translucent areas white, as in fig. 1, Pl. 24, and fig. 5, Pl. 25. The rings are probably not actually continuous, but, since they occur at about the same relative level in all the lobes of which the otolith is built up,

they present the appearance of complete rings parallel to the margin. These rings can be seen very clearly in fig. 1, Pl. 24, and fig. 5, Pl. 25. Their similar course in the lobes of which the plaice otolith is built may be seen in Immermann's fig. 6. As may be seen in fig. 5, Pl. 25, in the present paper, that part of the ring contained in an individual lobe is parallel to the curved border of that lobe. •

B. THE INTERNAL STRUCTURE OF THE OTOLITH.

The internal structure may be investigated by the examination of portions of otoliths rendered transparent by prolonged soaking in, for example, pyridine, or, better, by the cutting and grinding of thin sections. A thin transverse section of the uppermost otolith in fig. 1, Pl. 24, is shown in fig. 2, Pl. 24, orientated in its natural position. The Sulcus Acusticus is seen as a conspicuous indentation on the inner convex surface (to the right in fig. 2, Pl. 24), and on the same surface may be seen slight indentations, indicated by arrows, which are the concentric grooves on the surface mentioned in Section A. These grooves may also be faintly discerned on the opposite outer concave surface.

The most conspicuous feature in the section is a system of concentric lamellae, extending from the centre of the otolith to the periphery. The lamellae become thick where they cut the mid-sagittal plane of the otolith, becoming thin, almost to invisibility, in the mid-horizontal plane. The lamellae are not so clearly marked in the lower half of the section, because of the fractures about to be described.

The otolith of a small hake is slightly less flattened than that of a larger hake, but the general shape is unchanged during growth, and the otolith therefore grows more rapidly in length and depth than in breadth. Cunningham (1904) describes the similarly shaped lamellae in the plaice otolith as follows: 'each successive layer extends over the whole surface' (of a transverse section) 'but is exceedingly thin on the two flat surfaces, and thicker at the edge. The structure is such as would be produced if a sphere composed of concentric uniform layers of plastic

material were very much compressed so as to form a flat disk. The thin layers on the two faces being translucent, the surfaces of contact between successive layers are seen as lines approximately parallel to the outer edge'.

Immermann shows that an otolith comprises two constituents, namely, an inorganic crystalline constituent, and an organic fibrous constituent, which is a metamorphosed portion of the gelatinous connective tissue which fills the cavity of the sacculus. The present investigation shows that, of the two, the organic constituent is much the more important. They will now be described, as they occur in the hake otolith.

(a) *The Organic Constituent.*—When an entire otolith is carefully demineralized in weak acid, or, better, in Fol's solution, as formulated by Immermann, the organic constituent is left behind as a transparent, gelatinous and exceedingly brittle structure, which, though always much shrunken and distorted, has the identical lobed shape of the entire otolith. Moreover, the concentric shells (lamellae) described above can be discerned in the demineralized otolith, and are thus seen to belong to the organic constituent thereof. Unfortunately, as Immermann himself found, even with very gradual demineralization, gas-bubbles form within the otolith, and tear the very frail organic basis, which, also, always shrinks. In a demineralized otolith, therefore, the concentric shells are no longer arranged in the regular manner characteristic of the entire otolith.

The internal structure of the organic constituent may be investigated by the microscopic examination, either of demineralized sections of the otolith, or of serial sections cut from a previously demineralized otolith. The latter are preferable, because they may be cut into much thinner sections than those into which an entire otolith may be ground. On the other hand, it is exceedingly difficult to cut into serial sections a tissue so fragile as the organic constituent of the otolith, and the sections, when cut, break up very easily. Immermann's own photographs, in his figs. 3 and 4, of sections of the organic substance of a plaice otolith, bear eloquent witness of this difficulty! However, after many attempts, serial sections have been successfully cut, and stained with saffranin, as Immermann recommends.

A photograph of a portion of a section of the organic basis of a hake-otolith, cut transversely to the long axis of the otolith, and stained with saffranin, is shown in fig. 3, Pl. 24.

Three groups of concentric lamellae, cut transversely, are plainly visible, namely, at the top and middle of the photograph (*C.L.*) and at the bottom of the photograph. These are the thicker lamellae. Thinner lamellae are very faintly to be discerned, in the intervals between the thicker lamellae, as shadows or thin streaks. But the most conspicuous feature of the intervals between the thicker lamellae is the comparatively stout radial fibres (*R.F.*) which run from the centre of the otolith towards the periphery, and which pass, without interruption, through the concentric lamellae. In places (as to the right of the section) they are torn apart, probably by gas-formation during demineralization.

Under a very high magnification, the concentric lamellae tend to appear as rows of dots, as though they were themselves built up of a network of fibres running in a plane normal to that of the stouter radial fibres. Even under a lower magnification, as in fig. 3, Pl. 24, the concentric lamellae (*C.L.*) give this impression. The organic basis of the otolith seems to contain no structures other than the comparatively stout radial fibres, and the concentric shells, of varying thickness, which the radial fibres bind to one another.

As I have stated above, owing to shrinkage and distortion, a demineralized otolith is unsuitable for demonstrating the true spatial relations of the concentric shells to one another, or for showing how they vary in thickness. But they are very conspicuous in the entire otolith, as already described; and their true proportions may easily be shown in a thin section of an entire otolith. Such a section is shown, under high magnification, in fig. 4, Pl. 25. The photograph is one of that part of the section, shown in fig. 2, Pl. 24, which is marked with a cross.

In this photograph, which is taken near the vertical middle line of the otolith, we see, in the upper portion, a region of comparatively thick concentric lamellae (thick *C.L.*, marked with an arrow), in the middle, a region of extremely thin lamellae

(thin *C.L.*, marked with an arrow) which are just discernible in the photograph, and, finally, at the bottom left-hand corner, the commencement of a fresh region of thick lamellae. The lamellae, while varying so notably in thickness, appear to be spaced out at about equal intervals from one another, this distance being about 2μ . The thickest lamellae have a maximum thickness of about 1.5μ , the thinnest lamellae are almost ultra-microscopic, especially in those regions of the otolith in which their course runs parallel to the flat surfaces of the otolith, and where, therefore, as described above, they thin out. The photograph in fig. 3, Pl. 24, is of a region where the lamellae are nearly at their thinnest in their course around the otolith. As will be described below (Section C), however, the thin lamellae do not have quite the same shape as the thick lamellae, in that they do not thicken out at the vertical middle line to the same extent as the thick lamellae.

Fig. 4, Pl. 25, also gives some support to the hypothesis that the concentric lamellae are themselves compounded of a network of extremely fine fibres. If fig. 4, Pl. 25, is examined minutely, the thin lamellae, especially in the lower centre of the photograph, give the impression of being, really, rows of closely set fine dots, while the thick lamellae, especially in the upper centre of the photograph (about at the level of the upper arrow) tend to appear as rows of fine streaks. Presumably, in the latter case, the fine fibres are cut somewhat obliquely, for, owing to the curved course (parallel to the curved margin of the lobe) which the lamellae take in the lobes of which the otolith is built up (as described in Section A) any section of the otolith, which is not infinitely thin, will contain some or all of the lamellae cut obliquely.

The stout radial fibres, owing to the fact that they run parallel to the crystals which comprise the inorganic constituent of the otolith, are not to be seen in fig. 4, Pl. 25, unless the flecked or dappled radial streaks which are present everywhere in the photograph (and which are carefully to be distinguished from the fissures) are caused by them.

(b) The Inorganic Constituent.—Immermann states that the inorganic constituent of a plaice otolith consists of

needles of calcium carbonate, arranged in systems, and that a new system of needles seems to commence at each lamella. Maier describes the crystals in the otolith of the cod as fine little needle-crystals, and, in his fig. 9, he draws them as sharply pointed but not very slender.

Owing to the closeness with which the crystals interlock, it is exceedingly difficult to trace the outlines of any one crystal in the hake's otolith. It was soon realized that they must be minute, and therefore difficult to see for this reason also. If, however, the surface of a section of a hake otolith be etched for a short time with a demineralizing solution, small islets of crystals, which have been less affected by the reagent than others, are left standing out, and, by suitable focusing, some idea of the appearance and dimensions of the crystals in these islets may be obtained.

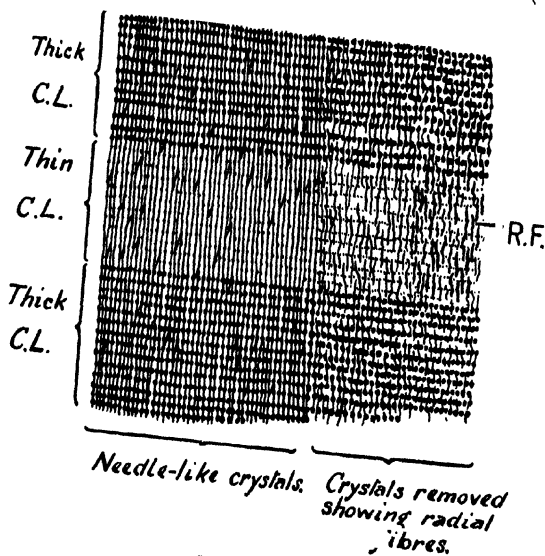
They are sharply pointed and exceedingly slender. If they are, indeed, entire crystals, they have a maximum length of about 40μ , and a width of less than 1μ .

Though the crystals are themselves so difficult to see, their arrangement in the otolith can be followed very easily by the course of the radial fissures which appear in every otolith section. They are to be seen among the solidly interlocking crystals clearly in fig. 2, Pl. 24, and are undoubtedly lines of cleavage produced by grinding. They are also well shown in fig. 4, Pl. 25 (Fissures). The fissures arise at the vertical middle line, and run out to the periphery in such a way as to cut through the concentric lamellae at right angles to them. The result of this is that those fissures which arise at the vertical middle line in the central region of the otolith run almost straight to the periphery, while those arising towards the upper and lower edges follow an increasingly curved course (fig. 2, Pl. 24). The two most important points about their arrangement in the otolith are, firstly, that they run through the lamellae normally to them, and, therefore, parallel to the radial fibres, and, secondly, that they are not in any way interrupted at the lamellae, but pass through them, nor is there any tendency for the lines of cleavage to arise at any particular lamella.

My views as to the microscopic structure of the hake otolith

may be summed up as follows, and are illustrated diagrammatically in Text-fig. 1.

The basis is a very frail basket work, consisting of sheets or networks of fibres, which form concentric shells (figs. 3,



TEXT-FIG. 1.

Diagrammatic representation of the relations of the constituents in the hake otolith. The lamellae are drawn as lines of dots placed between the slender pointed crystals. The right-hand part of the figure represents the structure of the demineralized otolith. *C.L.*, concentric lamellae; *R.F.*, radial fibres.

Pl. 24, and fig. 4, Pl. 25), bound together by stouter radial fibres (fig. 3, Pl. 24) and supported by needle-like crystals of calcium carbonate, which are orientated normal to the concentric lamellae and parallel to the radial fibres, and which interlock to form a very solid structure. In Text-fig. 1, the left-hand portion of the figure shows the columns of needle-like crystals, among which we must presume the radial fibres to run; the right-hand portion shows the same, demineralized, showing the radial fibres exposed by the removal of the crystals,

and rather shrunken. Crystals and fibres run normal to, and pass without interruption through, the concentric lamellae (*C.L.*), of which groups of thick lamellae are found in the upper and lower portions of the figure, groups of thin lamellae in the central portion. The lamellae are represented as rows of fibres passing between the crystals, in a plane normal to them, and therefore, cut in transverse section, appearing as dots.

One reason for the discontinuous or fibrillar structure of the concentric lamellae seems to be as follows. The concentric lamellae are perfectly definite structures, independent of the crystals, as any demineralized portion or section of an otolith, such as fig. 3, Pl. 24, clearly shows. The crystals are, also, perfectly definite structures, which are quite independent of the lamellae, running normally through them without interruption. It follows, therefore, that the lamellae, which obviously cannot pass through a crystal, must be interrupted in order to pass on either side of the crystal. I have endeavoured to show this, in Text-fig. 1, by placing dots, which represent the concentric lamellae, on the boundary lines between the crystals.

These views on the structure of the otolith are in good agreement, on the whole, with Immermann's. But Immermann did not observe the reticulate or fibrous structure of the concentric lamellae, whereas Maier, who observed neither the radial fibres nor the concentric lamellae in the otolith of the cod, describes, as layers filled with dark opaque granules, what are obviously the concentric lamellae, and he draws them as such in his fig. 9. He seems to have missed the very fine lamellae which, by analogy with the hake, occur between the thicker lamellae, but his observation clearly supports mine; and, further, Maier draws his crystals as running straight through the lamellae, without interruption, wherein, again, he supports my observation as against Immermann's.

The structure of the hake otolith, which is an ectodermal secretion, seems to be strikingly similar to that of another ectodermal secretion, namely, the nacreous layer in the molluscan shell, and the pearl. Lyster Jameson (1912) describes the nacre of the Ceylon pearl-oyster as follows: 'The organic basis which gives it its form, and which retains its iridescence after

the calcareous salts have been extracted, consists of a series of parallel lamellae, of extreme fineness, united to one another at intervals by radial connexions, so as to form a series of minute flat or lenticular chambers, separated by organic walls of extreme delicacy. The calcium carbonate appears to be enclosed in these chambers. . . . This structure is difficult to observe, owing to the distorting effect of the decalcification process, which, owing to the evolution of gas-bubbles, tears some lamellae apart, and forces others tightly together.' Amarthalingam (1929) publishes a photograph of a decalcified pearl, in which the concentric lamellae are clearly visible. Moreover, he very kindly sent me his preparations of pearl sections, which I was able to examine, and he has allowed me to state that, in the pearls of *Pinna*, at least, the fibres and crystals are arranged in a manner similar to that in the otolith of the hake.

C. THE CAUSE OF THE RINGED STRUCTURE.

The rings in the otolith of the hake have been described in Section A, and are portrayed in fig. 1, Pl. 24, and fig. 5, Pl. 25. In fig. 5, Pl. 25, are shown enlarged views of two otoliths, and beneath each is a photograph of a transverse section cut from it. A comparison of the sections with the entire otoliths shows that the more translucent rings, here appearing dark, correspond, exactly, with clear zones among the groups of concentric lamellae visible in the sections. This is confirmed by the examination of many other sections, not reproduced here. In my opinion, the rings visible in the entire otolith owe their greater translucency, undoubtedly, to the presence of these clear zones, which allow of an easier passage of light through the thickness of the otolith at the points where they meet the middle vertical line. The cause of the ringed structure, therefore, is to be found in the cause of the clear zones seen in the transverse section.

The fine concentric lamellae described, in Section B, as visible under very high magnification, tend to occur in groups, according to thickness, a group of thick lamellae being separated from the next group of thick lamellae by a few very thin

lamellae. The groups of thick lamellae themselves form, as it were, compound lamellae, which are plainly visible under low magnification—they can easily be seen in fig. 2, Pl. 24. The compound lamellae are, typically, in turn arranged in groups, such that there are regions where thick lamellae predominate, and but few thin lamellae are present, separated by regions where thin lamellae predominate, and thick lamellae are few or absent.

As has been stated, fig. 4, Pl. 25, is a photograph of the region marked X in fig. 2, Pl. 24, under high magnification. A region of thin lamellae occupies the centre of the field, while thicker lamellae occupy the upper and lower parts of the field. The region of thin lamellae appears as a transparent zone under a lower power of magnification (fig. 2, Pl. 24), while the thicker lamellae belong to the relatively opaque regions separated by the transparent zone.

The opacity produced by the groups of thick lamellae is comparable to that of the fat globules in milk, which, though they are themselves individually transparent, produce, in the mass, the effect of opacity and whiteness to the naked eye.

The distinctness of the rings seen in the entire otolith depends upon the sharpness of the grouping of the thick and thin lamellae. In a considerable proportion of otoliths, such sharpness is lacking in a part or the whole of the otolith, and an assessment of the number of the presumed 'annual rings of growth' becomes difficult or impossible.

Moreover, if the otolith is allowed to remain dry for any length of time, it becomes much more opaque, and translucency cannot then be restored by moistening. It is impossible to say whether this is due to an efflorescence of the crystals, or to a withering and shrinkage of the lamellae and fibres, or to the penetration of air bubbles. The plaice otolith, owing to its much smaller size, is still easily legible in such circumstances, but the bulkier hake otolith becomes almost useless.

Finally, the thin lamellae do not appear to thicken at the middle vertical line, at least as much as the thick lamellae. A succession of thin lamellae, therefore, leaves the edges of the otolith relatively more rounded than a succession of thick

lamellae; there is a slight change in the shape of the otolith as a whole. This may be seen distinctly in fig. 2, Pl. 24, where the transparent zones are notably more rounded at the middle vertical line than the opaque zones. When the region of thin lamellae becomes covered by a succession of thick lamellae, the slight change of shape causes a concave curvature of the succeeding lamellae, which is responsible for the grooves seen on the surface of the otolith. The grooves occur between the transparent zones, opposite the opaque regions of thick lamellae; but, if the course of growth is followed (in fig. 2, Pl. 24) by tracing the course of the fractures between the crystals from the periphery towards the centre of the section, the surface grooves will be found to correspond, in position, with the transparent zones.

Immermann considers that the lamellae have no part in causing the ringed appearance of the plaice otolith. He considers, however, that they cause, by some internal strain, a bending or twisting of the crystals. The plane of the crystals being slightly altered at the lamellae, there is a differential refraction and internal reflection of the light, causing zones of dark shadow. I cannot find, in the hake otolith, any sign of interruption of the crystals at the lamellae. The crystals, indeed, run straight through the lamellae from the middle vertical line to the periphery, as may be seen by following the course of the fractures in fig. 2, Pl. 24, and fig. 4, Pl. 25, without any bending or twisting. In any case, it is hard to imagine how any effect on the crystals could be produced by lamellae whose distance from each other appears to be not much greater than the width of the individual crystals, and much less than their length. An examination of sections of hake otoliths under polarized light, between crossed nicols, shows that the crystals are either continuous in their course through the lamellae, or are, at least, optically parallel.

Immermann supports his hypothesis, that the ringed structure is due to the inorganic rather than the organic constituent, by stating that the appearance of the otolith is not affected by the removal of the latter. He recommends two methods for removing the organic constituent, namely, gently warming in alkali,

and heating. I have repeated Immermann's experiments, made on plaice otoliths, with hake otoliths, and my results do not confirm his.

Small entire hake otoliths were warmed for months in strong caustic soda, frequently changed. The otolith was unaffected, except on the very surface, and it was clear that the penetration of the alkali was but slight. A thin section of an otolith, about 100μ thick, was therefore warmed in strong caustic soda for three months. The ringed structure was effaced, at least from the surface layers. Treatment with dilute acid removed the layers affected, and the rings once more became apparent. That the alkali, presumably by removing the organic constituent, destroyed the ringed structure, cannot be stressed, because the inorganic crystals were themselves seriously corroded by such drastic treatment.

The effect of heat has been tried on a great number both of entire otoliths and of sections. When an otolith is heated, there is, at first, a blackening, due to the charring of the organic matter, and in order to remove this completely it is necessary to use dull red heat. The otolith is thereby converted into an opaque, friable mass of lime, retaining the original shape, and even having the surface grooves unaffected. This mass is too brittle to grind down into sections, but if it be broken across, and the fractured surface be examined by reflected light, the original crystalline structure is seen to be represented by parallel columns of lime; but there is no sign of concentric rings, such as are easily seen in an untreated otolith examined in the same way, nor are there any signs of cracking or discontinuity among the columns of lime, corresponding to the concentric rings of the original otolith, such as Immermann claims to have found in the calcined plaice otolith. The result of this experiment is definitely adverse to Immermann's hypothesis.

It is true that the regularly zoned structure is destroyed by demineralization, but this would certainly result from the shrinkage and distortion of the very delicate organic basis of the otolith. As long as any crystals remain to support the lamellae, the typical structure persists.

SUMMARY.

1. The otolith of the hake (*Merluccius merluccius* Linn.) is a compound structure, comprising an organic and an inorganic constituent.

2. The organic constituent is the more complex in structure, and consists of concentric shells (appearing in sections of the otolith as lamellae) which have a reticulate structure, and are probably fibrous in nature. The concentric shells are separated at a fairly constant distance of about 2μ from one another, and are bound together by comparatively stout radial fibres.

3. The inorganic constituent consists of needle-like crystals, about 40μ in maximum length, and less than 1μ thick, which are secreted among the radial fibres, and pass normally through the concentric shells of the organic constituent from the centre of the otolith to the periphery. These crystals interlock to give the otolith its very solid structure.

4. The concentric rings, apparent when the entire otolith is viewed lying on its side, and which, in some species of fish, are used for the assessment of age, are due to the varying thickness of the concentric shells, described in (2) above. These are, typically, grouped in such a way that there are alternating zones where thick and thin concentric shells (lamellae) predominate. Where thick lamellae predominate an effect of opacity is produced, where thin lamellae predominate, one of comparative translucency. The thick lamellae are of the order of 1.5μ at their thickest, the thin lamellae extremely tenuous.

5. The cause of the ringed structure in the entire otolith is therefore due to the organic constituent, the inorganic constituent (crystals) having a supporting function only, in the otolith.

6. Immermann's hypothesis, that the rings are due to the structure of the inorganic constituent, being an optical effect produced by an internal reflection of the light, caused by a twisting of the crystals, is not accepted as applying to the otolith of the hake.

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DESCRIPTION OF PLATES 24 AND 25.

Photomicrographs taken with Edinger's Projection Apparatus (by Leitz).

C.L. Concentric lamellae. *R.F.* Radial Fibres.

PLATE 24.

Fig. 1.—Five hake otoliths, photographed by reflected light against a dark background. \times circa 2.

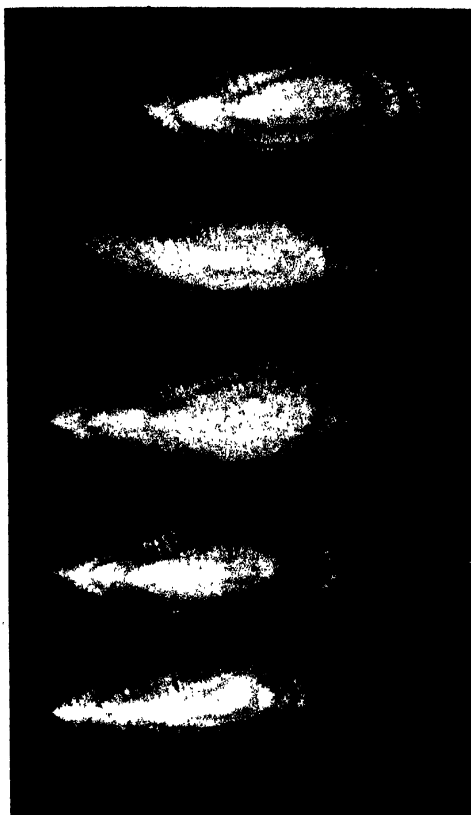
Fig. 2.—Section of the uppermost otolith in fig. 1, cut transversely to the long axis of the otolith, and ground thin. The arrows mark the surface-grooves described in the text. \times 18.

Fig. 3.—Portion of a section of a demineralized hake otolith (the organic constituent) cut transversely to the long axis of the otolith, and stained lightly with saffranin. Bausch and Lomb 4 mm. ocular, no. 5 eye-piece, using Wratten B light-filter. \times 313.

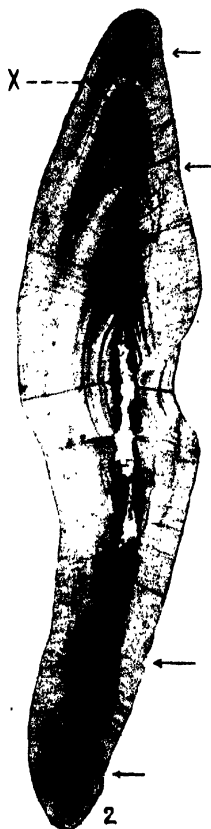
PLATE 25.

Fig. 4.—Portion of the transverse section of a hake otolith shown in fig. 2, in the region marked X, highly magnified. The conspicuous radial furrows represent lines of cleavage among the crystals. Zeiss $\frac{1}{12}$ " oil-immersion objective. \times 410.

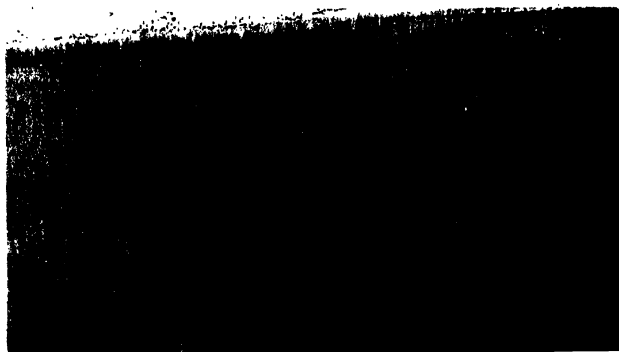
Fig. 5.—The anterior portions of two hake otoliths, photographed together with transverse sections cut from them. Otoliths and sections are reduced to the same magnification.



1



2



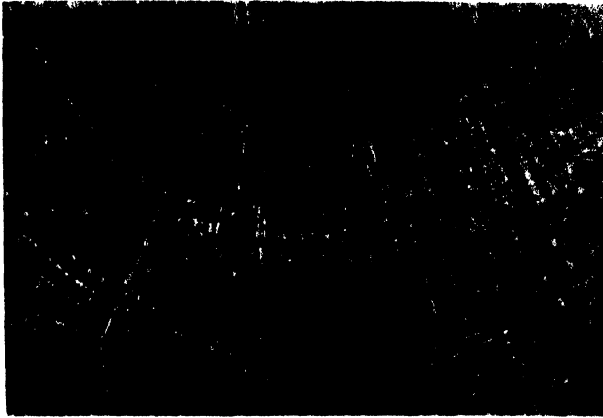
CL

RF

CL

RF

3



Thick C L

•
Thin C L

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Fissures

4



5

Studies on the Germ-cell Cycle of *Cryptocotyle lingua* Creplin.

1. Gametogenesis in the Adult.

By

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With 6 Plates (55 figures).

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I. INTRODUCTION.

THE germ-cell cycle is well known in only a few animals. Following the classical studies of Wagner (1862), Meinert (1864), and Metschnikoff (1865) on *Miastor* and those of Boveri (1887) on *Ascaris*, there have been more recent researches such as those of Wilson (1905) on the Hemiptera, Goldschmidt (1916) on *Lymnatria*, Schrader (1920) on *Trialeurodes*, Metz (1925) on *Sciara*, and Dobell (1925) on *Aggregata*. These results have stimulated investigation of the germ-cell cycle in both protozoan and metazoan forms.

In the parasitic flatworms the life-cycle is complicated by successive development in definitive and intermediate hosts and periods of asexual multiplication occur in which there are profound changes in organization and form. These complex phenomena have caused much dispute not only concerning the continuity of the germ-cell cycle, but also concerning the method of reproduction in the larval stages.

Many explanations have been advanced for the complicated life-cycles and modes of reproduction in the trematodes, but none has become generally accepted. Dollfus (1919) studied numerous distome and certain monostome species. He concluded that sporocysts, rediae, and cercariae do not develop from the body-wall of the sporocysts or redia; they come from one and the same germinal line which is derived from the cleavage of the fertilized egg. In the course of development of the individual, these germ-cells give rise to the larval somatic tissues of the sporocysts, rediae, and cercariae by a sort of internal polyembryony. The larval forms are only superimposed on the germinal line which is not interrupted from the fertilized egg to the adult trematode. The somatic tissues of the sporocysts and rediae are only larval envelopes which have enclosed the cells of the germinal line, and they do not take part in reproduction, but are sterile. Kathariner's (1920) conclusions concerning the development of the larval trematodes agree with those of Dollfus except that he believed polyembryony to be only apparent, not actual.

Brooks (1930) pointed out that metagenesis, paedogenesis,

metamorphosis extending over several generations, dissogeny, and heterogony, all of which have been advanced at one time or another to account for the asexual phases of the life-history, cannot be accepted as explanations of the germ-cell cycle in the digenetic trematodes. According to Brooks, metagenesis implies that an hermaphroditic adult generation alternates with one which reproduces asexually; paedogenesis requires a functionless adult generation; metamorphosis lacks the necessary cytological evidence; dissogeny and heterogony require maturation of the reproductive cells giving rise to cercariae. Reuss (1903), Haswell (1903), Tennent (1906), Cary (1909), and Faust (1918) have reported finding polar bodies among these cells, while Looss (1892), Coe (1896), Rossbach (1906), Dollfus (1919), and Mathias (1925) have either failed to find them or have not interpreted them as such. Brooks (1928) also failed to find polar bodies, stating, 'The formation and dissociation of the germ-masses is considered to be a typical case of polyembryony. . . . This factor is operative first in the miracidium whose somatic development to form a typical adult is restrained by the early impetus of germinal multiplication so that it becomes a "mere reproductive sac" after entering its snail host. The germ-cells within this miracidium-mother-sporocyst stage undergo a similar process to form mother rediae or sporocysts. The same process is repeated as many times as there are intercalary stages, stopping only when the cells of the germinal line have attained sufficient physiological age and morphological differentiation to produce a form (the cercaria) in which the germ-cells are normally restrained and in which the somatic elements can therefore go on to develop a true adult'.

The trematodes vary greatly among themselves in regard to the intercalary stages of their life-histories. *Fasciola hepatica* has sporocysts, rediae, daughter rediae, and cercariae. *Cryptocotyle lingua*, with whose germinal development this paper is concerned, has no daughter rediae and the attainment of sexual maturity is deferred by a meta-cercarial stage. *Schistosoma japonicum* has no redial stages, the cercariae develop in sporocysts, and the sexes are separate. The sporocysts of *Cercariae Indicae* XV

(Sewell, 1922) produce miracidia as well as cercariae. *Leucochloridium* has no redial or free-living stages.

Since the life-history of *Cryptocotyle lingua* is now well understood (Stunkard, 1930) and material is easily obtained, it seemed desirable to make a study of gametogenesis in the adult trematode and of the mode of reproduction in the larval stages in order to secure information bearing on the germ-cell cycle in the Trematoda. The present paper will deal only with spermatogenesis and oogenesis in the adult. Although significant observations have been made on larval reproduction, the study is not as yet complete; it will be the subject of a later report in which the much disputed question of larval development in the trematodes will be discussed.

The writer is greatly indebted to Dr. Horace W. Stunkard, who suggested and directed this study. He is also grateful to Dr. C. H. Willey, who very kindly gave valuable assistance in matters of cytological technique and photomicrography.

II. HISTORICAL SURVEY.

The studies which have been made on gametogenesis and fertilization in the adult trematodes are not extensive. Most of these investigations have been concerned either with spermatogenesis or oogenesis (rarely both). Goldschmidt (1902) described oogenesis in *Polystomum integerrimum*, a monogenetic species in which the diploid chromosome number is eight. He did not state whether the first or second maturation division is reductional. Henneguy (1902) studied oogenesis, maturation, and fertilization in *Distomum (Fasciola) hepatica*. He described the formation of 'ectolecithal' eggs in this species and a condition in which spermatozoa not needed for the actual fertilization process were absorbed by the vitelline cells. His account of maturation was not complete, since he observed only a few of the stages. Von Janicki (1903) observed certain stages of oogenesis and cleavage in the eggs of *Gyrodactylus elegans*, a monogenetic species. He counted eight chromosomes as the diploid number, but expressed some doubt as to the correctness of this observation. Kathariner (1904) made a further study of oogenesis, fertilization, and

cleavage in *Gyrodactylus*. He also reported that eight is the diploid chromosome number and that this number appears before the first maturation division. He did not state which division was reductional. In a later investigation of oogenesis, fertilization and cleavage in *Gyrodactylus*, Gille (1914) has maintained that the diploid chromosome number is twelve instead of eight, and that the chromosomes appear as six dyads in the equatorial plate of the first maturation spindle. He did not observe a longitudinal splitting of these (dyads), nor did he definitely state where reduction occurs.

In *Zoogonus mirus*, Goldschmidt (1905) counted ten chromosomes and described a peculiar scheme of maturation (Primärtypus). His observations, however, have been disputed by A. and K. E. Schreiner (1908), Gregoire (1909), and Wassermann (1913), all of whom studied oogenesis in *Zoogonus mirus*. They maintained that the diploid chromosome number is twelve instead of ten, that the first maturation division is reductional, that the haploid number of chromosomes appear as bivalents before this division, and that, consequently, the 'Primärtypus' of Goldschmidt does not exist. Actually, the Schreiners used Goldschmidt's own preparations but were unable to confirm his findings. Goldschmidt (1908) furthermore described oogenesis and fertilization in *Dicrocoelium lanceatum*. According to him, this form has a diploid chromosome number of twenty and reduction occurs at the first maturation division. Schellenberg (1911) counted twelve chromosomes as the diploid number in *Fasciola hepatica*. He reported that in oogenesis pseudoreduction occurs previous to the first maturation division and that this division is reductional. Schubmann (1905), studying oogenesis in *Fasciola hepatica*, described a condition in which part of the oocytes degenerate and their degeneration products provide nourishment for the remaining cells. Schellenberg (1911) also observed intercellular granules or degenerating cells in the ovary of *Fasciola hepatica*, but did not believe them to be present in sufficient numbers to provide a means of nourishment. He stated, 'Ich bin daher nach diesen Befunden der Ansicht dass die Keimzellen des Ovars durch von aussen geliefertes Secret

ernährt werden und nicht ihre Existenz auf den Zerfallsprodukten ihrer Schwesterzellen aufbauen'. In *Gyrodactylus elegans*, Kathariner (1904) had described nutritive cells in the cytoplasm of the oocytes. Gille (1914), studying the same species, found that the oocyte is accompanied by one or more nutritive cells when it passes from the ootype to the uterus. He stated that these cells are abortive oocytes whose degeneration products are distributed throughout the nucleus of the chief oocyte by means of pseudopodia.

Dingler (1910), working on *Dicrocoelium lanceatum*, made the first definite study of spermatogenesis in the trematodes. Later workers, studying other species, have been unable to confirm his observations of the mitochondria. Dingler described intercellular granules in the testes of *Dicrocoelium* and suggested that they were probably nutritive in function.

Von Kemnitz (1913) described gametogenesis and fertilization in *Brachycoelium salamandrae*. He is the only worker who has studied both spermatogenesis and oogenesis in the same species of hermaphroditic trematodes. He counted twenty chromosomes in this form and observed pseudoreduction previous to the first maturation division which he believed to be reductional. His account of spermatogenesis, however, is incomplete, since he followed the process only to the first spermatocyte division.

Lindner (1914) studied spermatogenesis in *Schistosoma haematobium* and gave the first account of sex chromosomes in the dioecious genus *Schistosoma*. Eight chromosomes appear before the first or reductional maturation division; six of these are bivalent while the remaining two are univalent 'Heterochromosomen'. The bivalents separate into two groups of six univalents each. The two 'Heterochromosomen' or sex chromosomes pass to one of these groups and the second division is equational. As a result, there are two types of spermatozoa, one having six and the other eight chromosomes. Sex determination occurs at the time of fertilization of the egg which always possesses eight chromosomes. Severinghaus' (1927) description of gametogenesis in *Schistosoma japonicum* corrected the earlier account of Faust and Meleney (1924) who

maintained that the two types of spermatozoa in this species have seven and eight chromosomes respectively. This would give an important species difference between *Schistosoma haematobium* and *Schistosoma japonicum*. Severinghaus, however, found that the males have fourteen chromosomes and that there are six chromosomes in the male-determining, and eight in the female-determining spermatozoa. The females possess sixteen chromosomes and are homozygous, since they produce only one type of ova. Consequently, sex is determined by the spermatozoa at the time of fertilization, males having twelve somatic and two sex chromosomes and females having twelve somatic and four sex chromosomes. Severinghaus has produced experimental evidence to show that the miracidium is sex determined and can give rise only to sporocysts and cercariae of its own sex.

The genus *Schistosoma* is dioecious, however, and there are obvious possibilities for differences in chromosome behaviour between the dioecious and hermaphroditic trematodes. Since the zygote of the hermaphroditic form must give rise to both ovaries and testes, there is no reason to expect two types of either spermatozoa or ova.

III. METHODS.

The adult worms were taken from the intestine of the tern at Woods Hole in the summer of 1929, fixed in Helly's solution, and preserved in 70 per cent. alcohol. The specimens were sectioned at four micra and fixed for ten minutes on the slide in Flemming's strong solution for the purpose of improving cytological differentiation. Heidenhain's iron-alum haematoxylin staining proved best for chromatic structures. The safranin light green method was used in search of achromatic elements. Spencer 10 \times and 15 \times oculars and 1.8 and 1.5 apochromatic objectives were used in this study. Photomicrographs were made with the Zeiss apparatus, using Eastman medium iso and process plates.

It should be pointed out here that the histological structure and the degree of staining varies greatly with the age or development of the organism. In the less mature specimens the gonads

are compact, the body tissue possesses fairly definite organization, the intestine is easily recognized in the sections, and the karyosomes of the cells stain intensely with iron-haematoxylin. Chromatin granules and spireme threads are barely visible, even when the process of differentiation (destaining) is not carried far. The karyosomes appear to be of homogeneous structure. In the mature worm the tissues are loosely organized, the gonads are not compact, and spireme threads stain intensely, while the karyosomes take a faint stain and show clearly that their structure is not homogeneous. This difference in staining cannot be due to variations in staining technique, since the specimens were imbedded in clumps and worms of all degrees of sexual maturity were mounted on the same slide and subjected to the same technique. Under these conditions the difference is very striking.

IV. OBSERVATIONS.

The Chromosomes of *Cryptocotyle*.

One of the first objects sought in the present study was the determination of the number, size, and shape of the chromosomes and their behaviour in division processes. Mitotic figures from which the number can be determined at all accurately are very scarce. The cells are small and divisions are not frequently observed in somatic and ovarian tissues. This would seem to indicate that the metacercariae become practically mature in regard to somatic and ovarian cell numbers. At certain stages, mitotic figures are relatively plentiful in the testes, but the cells are even smaller than they are elsewhere. Consequently, chromosome counts in adult worms are difficult to obtain. The first definite count of the chromosomes was determined from a mitotic figure found in the somatic tissue near the posterior end of a young worm. Figs. 4 and 5, Pl. 26, represent the chromosomes as they appeared in this cell, distributed through two sections. This number, twelve, has been verified once at the division of the primary spermatogonia (fig. 9, *a* and *b*, Pl. 26), and six chromosomes have been observed many times in the haploid condition previous to and after the

meiotic division. Fig. 51, Pl. 31, clearly shows these as bivalent chromosomes prior to the reductional division of the oocyte. Fig. 2, Pl. 26, represents the chromosomes separated⁷ and arranged in pairs. There are two pairs of larger chromosomes, one of which is U-shaped with arms of about equal length, while the other pair is more V-shaped with the arms unequal in length and considerably thicker than the angle. These larger chromosomes are about five micra in total length. There are four pairs of smaller chromosomes, three of which are bilobed. Two pairs of these bilobed chromosomes resemble diplococci, while the third pair possesses lobes which are unequal in size. The remaining pair appears to be rod-shaped. With the exception of this pair, which is slightly larger, all of the smaller chromosomes are about one micron in their greatest dimension. In mitosis, these precede the larger chromosomes in moving to the poles of the spindle. Astral rays, centrosomes, or spindle-fibres have not been observed satisfactorily. The stages of mitosis in the somatic tissues are essentially the same as those found in other animals. Figs. 1 and 3, Pl. 26, show an anaphase and a prophase of somatic mitosis. The resting cell may have one large karyosome, one or more smaller karyosomes in addition to a larger one, or, in some cases, two of nearly equal size.

Spermatogenesis.

The Testes.

The testes are two in number, located in the posterior end of the worm. They are approximately 100 micra in diameter in sexually mature specimens, and are irregularly oval in shape. In immature worms, they are very compact and usually exhibit various stages of spermatogenesis near the centre. The walls are composed of several layers of cells, some of which may be in various stages of division. In mature worms, the testes are loosely organized with the clumps of germ-cells considerably separated. Strands of connective tissue and residual cytoplasmic masses (see below) are found in the open spaces of the gonad. The wall is only one cell thick in some cases. In others it is scarcely recognizable at all, since the cells are quite a distance apart and its identity is maintained only by connective

tissue. Figs. 48 and 49, Pl. 30, are photomicrographs of rather immature testes. Fig. 6, Pl. 26, and fig. 50, Pl. 31, represent a more mature testis. Spermatogenesis undoubtedly precedes oogenesis in *Cryptocotyle*, since the testes attain a considerable degree of maturity before ova are found in the ootype.

The Primary Spermatogonia.

The testes of the immature worm consist largely of closely packed cells. Their nuclei usually contain two karyosomes. These are the primitive germ-cells ('Ursamenzellen' of Dingler, 1910), and are, therefore, the primary spermatogonia. Their nuclei may exhibit any stage of mitotic division. If several dividing cells are present and adjacent to one another, one cannot be certain that they are primary spermatogonia. In such cases they may be confused with later stages of spermatogenesis. Their position in relation to the wall of the testes is of much significance, however, since the primary spermatogonia are located near the edge of the gonad as the worm becomes more mature. Figs. 7 and 8, Pl. 26, represent primary spermatogonia in the resting and spireme stages respectively.

The Secondary Spermatogonia.

After a period of multiplicative division, the primary spermatogonia divide to form two-cell clusters (fig. 10, Pl. 26), that is, the daughter cells tend to remain together. These are the secondary spermatogonia. Fig. 9, *a* and *b*, Pl. 26, represents, in two sections, the prophase of the primary spermatogonial division. The diploid chromosome number, twelve, can be clearly ascertained in this case. The association of the secondary spermatogonia in groups of two is more clearly seen in the mature worm. In younger specimens, the only reliable identification of the secondary spermatogonia is the finding of two adjacent cells in the same stage of division. Of course it is possible that contiguous primary spermatogonia may divide simultaneously. The secondary spermatogonia also tend to remain near the wall of the testis in more mature worms. They usually possess a single karyosome which stains intensely in young worms, but is often hard to distinguish in mature specimens.

The Tertiary Spermatogonia.

After a resting period, the secondary spermatogonia divide to form four-cell clusters (fig. 12, Pl. 26), the tertiary spermatogonia. Fig. 11, *a* and *b*, Pl. 26, was taken from the division of a secondary spermatogonium found in two sections. The other cell of the cluster was in the same stage, but the chromosomes were not so favourably arranged for drawing. Owing to the clumping of those in fig. 11, Pl. 26, it was impossible to definitely count more than eleven chromosomes. Part of one V-shaped chromosome was found in each section. The tertiary spermatogonia, like the secondary spermatogonia, may be recognized by their division stages. The tertiary spermatogonia tend to lie nearer the centre of the testis, although in some cases, especially in older worms, they remain with at least part of the cells of the cluster forming the outermost layer of the wall. They ordinarily have a single karyosome (fig. 12, Pl. 26) and undoubtedly possess the diploid number of chromosomes (fig. 11, *a* and *b*, Pl. 26) although definite counts of twelve have not been made.

The Primary Spermatocytes.

The tertiary spermatogonia divide (fig. 13, Pl. 26) to form an eight-cell cluster, the primary spermatocytes (fig. 14, Pl. 27). Only five of the eight cells are seen in this section. Definite chromosome counts have not been made at the division of the tertiary spermatogonia to form primary spermatocytes on account of the small size and clumping of the chromosomes. The larger chromosomes, however, have made it possible to ascertain that the diploid chromosome number is possessed by the primary spermatocytes. (Fig. 13, Pl. 26, represents the prophase of the tertiary spermatogonial division which gives rise to the primary spermatocytes.) In most cases the primary spermatocytes are found separated from the wall of the testis. They stain faintly in young worms and intensely in older specimens. They have a single karyosome which is readily seen, fig. 48, Pl. 30.

Reduction.

In the division of the primary spermatocytes reduction occurs. In the resting cells (fig. 14, Pl. 27) the chromatin is scattered evenly throughout the nuclei. This condenses into threads which fuse to form a filament which may be continuous although such a continuity has not been traced. In the oocytes, leptotene threads of considerable length have been followed. In the primary spermatocytes the threads have a double appearance before and after they break up to form loops. Syndesis follows with a typical bouquet formation. Fig. 15, *a* and *b*, Pl. 27, represent two cells from a cluster of spermatocytes which were observed at this stage. A careful study was made of the cell represented by fig. 15 *a* in order to determine if possible the number of loops in this bouquet. The manner in which they cross one another as well as the possibility that the point of condensation may contain small loops has made this a most difficult task, and although six have been definitely traced, it is not impossible that there are more. If twelve were present, however, it seems more than likely that a greater number than six would be easily seen. Fig. 15 *b* represents another cell from this same cluster as seen from above. Here it would be impossible to make a definite count, although more than six appear to be present. It may be that a single loop extends the entire width of the cell, and, as seen from above, resembles two loops on account of the tangled condition at the centre and the condensation beneath. It was impossible to distinguish a doubled condition of these loops. Condensation increases until a dark irregular mass is formed. It is believed that the chromosomes are formed directly from this mass without the intervention of a diffuse stage as found in the oocytes. At any rate, pseudo-reduction occurs in which the haploid number of bivalent chromosomes appears. They can be easily counted and are clearly seen to have retained their characteristic proportions and shapes, but are larger in size than the chromosomes of any of the previous divisions. The univalent units of these bivalent chromosomes then begin to separate. Figs. 17, 18, and 19, Pl. 27, show various stages of this separation. Fig. 16, Pl. 27,

represents one section containing three of a group of eight reduction divisions. The process seems to start in the larger chromosomes by a splitting near the ends. This may be accompanied by a straightening of the curved chromosomes (fig. 19, Pl. 27). This condition has been observed many times. There may be a considerable lengthening of them. It has not been proved that the loops (fig. 19, Pl. 27) clearly visible at a later stage are due to a twisting of the separating parts about one another, but their appearance in certain instances, especially the thickening nodes at the ends of the loops, suggests strongly that this is the case. The shapes of the smaller chromosomes seem to be reflected in the manner in which their univalent components separate. The ones resembling diplococci seem to separate evenly through the centre. They remain fused at each end, and open out into rings at a later stage. The divisions of the others are more uneven. Figs. 48 and 49, Pl. 30, are photographs showing the actual splitting of the bivalent chromosomes (*rd*).

The Secondary Spermatocytes.

The sixteen-cell clusters resulting from the reduction division of the primary spermatocytes are the secondary spermatocytes. In young worms these stain faintly in the resting stage (fig. 20, Pl. 27), but it is easy to observe the sixteen clusters of chromosomes in the early stages of the ensuing division (fig. 22, Pl. 27). Only seven groups are observed in this section. The resting secondary spermatocytes stain intensely, however, in mature worms. They have a single karyosome and are usually found well away from the wall of the testis unless the worm is very mature. Fig. 21, *a*, *b*, and *c*, Pl. 27, are some condensation figures that have been observed previous to secondary spermatocyte divisions.

The Spermatids and Spermatozoa.

The division of the secondary spermatocytes (fig. 22, Pl. 27) results in a thirty-two-cell cluster, the spermatids (fig. 23, Pl. 27, and fig. 50, Pl. 31). These have the same staining characteristics as the secondary spermatocytes. There now

begins a gradual elongation of the nuclei accompanied by a condensation of the chromatin. The spermatids are at first ovate with the pointed end drawn out and directed towards the centre of the cluster (fig. 24, Pl. 27). As they become longer and thinner, they stain more intensely and lose their granular appearance (fig. 25, Pl. 27, and fig. 26, Pl. 28). They then become twisted into a confused mass which later seems to become orientated in respect to the vas deferens (figs. 27 and 28, Pl. 28). One end of the spermatid, which is now threadlike and may be termed a spermatozoan, is drawn out while the other remains in a tangled coil. The spermatozoa may straighten out entirely as they move into the vas deferens, although they are frequently found in the seminal vesicle with their ends still coiled. The spermatozoan does not appear to have a differentiated head or other specialized parts such as are found in the male gametes of many animals. It is long, threadlike, apparently of uniform width, and tapers to the ends. In the later description of oogenesis and fertilization additional information is given concerning the nature of the spermatozoan.

Cell Boundaries and Residual Masses.

In the foregoing description of spermatogenesis in *Cryptocotyle* no mention has been made concerning cell-boundaries in the various stages, or the ultimate fate of the cytoplasm. The mature worm must be studied for these observations: In the spermatogonial and primary spermatocyte stages the cell-boundaries are usually quite distinct. In later stages, however, only traces of cell-boundaries can be observed. In cross-sections of young spermatid clusters there are darker strands or zones extending radially through the cytoplasmic 'matrix' from the centre of the syncytium, if so it be, to the individual spermatid nuclei (fig. 6, Pl. 26, and fig. 23, Pl. 27). The nature of these zones is unknown, but it is suggested that they have something to do with the elongation of the spermatids, since they lengthen in a direction extending toward the centre of the cluster. It appears, then, that only the nuclei go to form the spermatozoa, while their cytoplasmic material is left behind in the form of what Dingler (1910) called an abandoned residual

mass. If the spermatozoan is entirely nuclear, then, an explanation may be suggested for its uniform structure and the fact that the entire body of the gamete apparently enters the egg.

Oogenesis.

The Ovary.

The single ovary is located on the opposite side and in front of the anterior testis. It is about the same general shape as the male gonads, a little more elongate, and about 100 micra in its greatest dimension. There is not as much variation in the appearance of the ovary in animals of different ages as there is in the testes. Staining reactions resemble those described in spermatogenesis. In the more mature worms the karyosome appears to have a clear vesicular centre with a more or less reticular peripheral structure (fig. 29, Pl. 28, and fig. 52, Pl. 31). In immature specimens it stains uniformly and intensely as a rule. The cells near the edge of the ovary are smaller and usually have two karyosomes of about equal size, although this condition is not without exception. Those nearer the centre exhibit various appearances which are described below.

The Oogonia and Oocytes.

A definite line of demarcation of the oogonia from the oocytes in the ovary of *Cryptocotyle* does not exist. They may be distinguished, sometimes with difficulty it is true, by their size, which is much smaller in the case of the oogonia and the number and size of their karyosomes. The oogonia usually possess two karyosomes of about equal size. They closely resemble primary spermatogonia. The oocytes possess one large or one large and one small karyosome as a rule. The oogonia are located near the wall of the ovary, and are spherical to oval in shape, the nuclei being about 6 micra in diameter. The oocytes are found nearer the centre of the ovary, and are of the same general shape as the oogonia, the nuclei being about eight micra in diameter. In some specimens almost all of the oocytes show minute deeply staining bodies, one in the cytoplasm of each oocyte, very near or touching the nuclear membrane. If these are not of the nature of centrosomes, it seems difficult to account

for them, although they have not been of constant appearance in the preparations. The oogonia are usually observed in the resting stage with the chromatin rather uniformly distributed throughout the nucleus. They divide occasionally by simple mitosis. Such divisions are not often observed. This would indicate that most of the period of multiplication occurs in the metacercariae or very young adults, or that the process of division itself, when once started, proceeds very rapidly. Sufficient observations of oogonial division have been made, however, to make it quite certain that the chromosome number at this stage is diploid, although definite counts of twelve have not been obtained from the cases observed. The oogonia increase in size and differentiate to form oocytes. There is a considerable loosening in the texture of the ovary as the worm becomes more mature, but this condition never becomes as conspicuous in the ovary as in the testes.

Pre-meiotic Phenomena in the Oocyte before Sperm Penetration.

Before the oocyte leaves the ovary and passes into the ootype, a preparatory process for the later reduction division occurs. A number of stages may be observed among the oocytes. Fig. 29, Pl. 28, and fig. 52, Pl. 31, show typical resting oocytes. The rather uniformly distributed chromatin condenses to form nodular strands (fig. 30, Pl. 28). These seem to condense farther and appear double (figs. 31 and 32, Pl. 28). This doubled condition is probably the result of two strands coming to lie side by side. The thread has not been proved to be continuous, since an entire oocyte was never contained in a single section, but threads of considerable length have been traced and it is believed that there is a continuous double thread at one stage. In the next stage the thread is broken into pieces of various lengths (fig. 33, Pl. 28). Syndesis follows with typical bouquet formation (fig. 34, Pl. 28, and fig. 52, Pl. 31). This stage is very scarce in the preparations. It is followed by a diffuse condition in which it remains until penetration of the spermatozoan. The stages found in this study have given strong indication of doubling by parasyndesis.

Nutrition of the Oocytes.

In this study, groups of two or more nuclei resembling those of oocytes and degenerating masses have been observed in the ootype, embedded in a common cytoplasmic matrix. Several stages of this condition have been found. Fig. 36, Pl. 28, represents one in which there are two large nuclei and a degenerating mass, presumably from a third nucleus. Fig. 37, Pl. 28, shows a similar case in which one nucleus has four small karyosomes while the other has a single large one (in preceding section). Fig. 35, Pl. 28, is still another case in which all the nuclei except one have become much reduced. This condition had been described in *Gyrodactylus elegans* by Kathariner (1904) and Gille (1914). The latter believed these nuclei which degenerate to be those of abortive oocytes whose disintegration products nourish the chief oocyte. All but one of the nuclei do degenerate, no doubt, and their materials probably nourish the remaining oocyte. There is a possibility, however, that these nutritive cells are not abortive oocytes. They may be vitelline cells which have the same origin embryologically as the oocytes, and except for their peripheral yolk droplets, resemble oocytes very closely. It is known that these cells move into the ootype and that they ultimately degenerate. It is possible that one or more of them may fuse with the single oocyte as it leaves the oviduct and then degenerate, nourishing the oocyte. The oocytes have been observed in clumps in the ovary, but in practically all cases there have been too great a number in one of these groups to believe that all but one degenerate. Besides, smaller or disintegrating oocytes have never been observed in these clumps while within the ovary. At the time of sperm penetration and shell formation there are no traces of these nuclei in the cytoplasm of the oocyte. The pseudopodia described by Gille (1914) were not observed in this study. It is thought that these may be due to collapse of the nuclear membrane during fixation, since cytoplasmic pseudopodia extending into a nucleus is most unusual. Whatever their source, however, the degenerating nuclei are probably nutritive in function.

Sperm Penetration and Shell Formation.

The nucleus of the oocyte is in a state of rest when it comes in contact with the spermatozoan. Fig. 38, Pl. 29, represents the oocyte at this stage. The spermatozoan first appears to wrap itself around the oocyte and then the entire gamete apparently penetrates the cytoplasm. After this process, the yolk material collects around the oocyte and the shell of the egg is formed (fig. 40, Pl. 29). The yolk droplets may unite, forming one or more large refractile globules (fig. 46, Pl. 29). These are very conspicuous. The remaining nutritive material appears as clumps of granules. The egg cell lies near the opercular end of the shell, while the yolk material is more localized at the opposite end (fig. 53, Pl. 31). The shell is light yellow at first, but later becomes darker in colour. Globules of shell material are abundant among the vitelline cells.

The First Maturation Division.

The observation of maturation in the oocytes of *Cryptocotyle* is very difficult because of the opacity of the shell and the difficulty of sectioning the eggs. Perfect serial sections of the ovum are extremely difficult to obtain, owing to the tendency of the knife to either skip or catch in the shell and damage the sections. For this reason, favourable preparations are largely a matter of chance. By sectioning several hundred specimens, however, enough stages have been observed to make it possible to give a fairly complete account of the maturation processes. The precise phenomena concerned with the appearance of the six bivalent chromosomes of the first maturation divisions have not been fully observed, but condensation stages have been seen in disconnected cases (figs. 41 and 42, Pl. 29, represent two of these). Fig. 39, Pl. 29, shows a condition which was observed only once. Here the shell had not been formed although yolk material surrounded the oocyte. The chromatin of the nucleus had condensed to a considerable extent and the proportionate amount of cytoplasm was greater in this case than in any other observed. It has seemed best, however, to give most stress to the fact that, regardless of how the chromosomes are formed from the diffuse stage, they appear before the first maturation

division in the reduced or haploid number and, consequently, pseudoreduction is typical. Fig. 43, Pl. 29, and fig. 51, Pl. 31, show the bivalents as they appear before the first maturation division. Meanwhile, the sperm (figs. 41, 43, and 44, Pl. 29, and fig. 51, Pl. 31) shortens and thickens until it is almost comma-shaped at the beginning of the first polar division. Each of the six bivalents split, presumably along the line of fusion during syndesis. Hence this division is reductional and the resulting cell possesses the haploid number of monovalent chromosomes. The count has been verified at this stage (fig. 51, Pl. 31) and the process of reduction is very similar to that occurring in spermatogenesis of *Cryptocotyle*. Fig. 46, a and b, Pl. 29, represents the anaphase of the first maturation division of a single egg which was found in two sections.

The Second Maturation Division.

The structure of the chromosomes and the sperm is the distinguishing characteristic of the second maturation division. Figs. 53 and 54, Pl. 31, well illustrate this. Here we see that the chromosomes are more filamentous than in the first or reductional division, where they are thick and rather closely resemble those found in somatic mitosis. The first polar body can be seen as a small shadowy mass near the sperm nucleus in fig. 54, Pl. 31. The sperm is spherical at this stage and has a looser, granular appearance. Since it is assumed that the first is reductional, the second maturation division must be equational.

The Pronuclei and Fertilization.

Following the second polar division, there is apparently a reorganization of the egg cell, with the appearance of the female pronucleus. The sperm increases in size and closely resembles the female pronucleus. Fig. 47, Pl. 29, shows a stage which has been interpreted to be the male and female pronuclei with the second polar body. This stage resembles the oocyte with its nutritive cells (fig. 36, Pl. 28) and could be interpreted as such if it occurred in the ootype before the yolk granules were deposited and the shell was formed. Each nucleus has a single large karyosome and rather uniformly distributed chromatin.

In this case, the first polar body was not observed. It evidently had either degenerated or left the cytoplasm of the egg and mingled with the yolk material, where it would be almost impossible to recognize it. Fig. 45, Pl. 29, represents a stage which has been interpreted as the fertilization stage because of its different appearance, apparent diploid condition as to the number of chromosomes, and the absence of a sperm.

Cleavage.

In this study little attention has been given to cleavage and miracidium formation, but it has been observed that the young larva is in a fairly late morula stage when the egg escapes. It is quite likely, nevertheless, that there is great variation in the degree of development of the young larvae when they leave the body of the parent worm. Fig. 55, Pl. 31, represents a two-cell stage of cleavage. Further observations of the development of the zygote to form the miracidium will be very difficult until some manner of removing the shell without injurious effects is perfected.

V. DISCUSSION.

The present study has shown that gametogenesis in *Cryptocotyle lingua* is similar in its general aspects to that described by various workers for other species of trematodes. The stages of spermatogenesis in *Cryptocotyle* resemble those described by Dingler (1910) for *Dicrocoelium lanceolatum*. The successive doubling of the nuclear number in the germ-cell clusters, the differentiation of the spermatids to form spermatozoa, and the abandoned cytoplasmic masses, as observed by Dingler, were found to be essentially the same. Von Kemnitz's (1913) description of the reduction division in the spermatocytes of *Brachycoelium salamandrae* is similar to that in *Cryptocotyle*, particularly in regard to the behaviour of the separating components of the bivalent chromosomes. He did not describe the stages subsequent to this division, however, and his account is consequently incomplete. The only other significant studies have been made on spermatogenesis in dioecious trematodes; those of Lindner (1914) on *Schistosoma haematobium*, and of Faust and

Meleney (1924) and Severinghaus (1927) on *Schistosoma japonicum*. It is evident that there may be considerable difference between spermatogenesis in the schistosomes and in *Cryptocotyle*, since the former are dioecious and the latter are hermaphroditic.

The findings concerning oogenesis in *Cryptocotyle* cannot be harmonized with Goldschmidt's (1905) conclusions concerning the so-called 'Primärtypus' of maturation in *Zoogonus mirus*. He gave the following schemes of chromatic behaviour in the maturation of different animals.

- I. 'Primärtypus'; no pseudoreduction; normal number of chromosomes appear in the nucleus; these divide in one of the maturation divisions. *Zoogonus*.
- II. Pseudoreduction; the haploid number of chromosomes appear. Two types:
 1. 'Tetradentypus'; tetrads that are
 - (a) fused entire chromosomes. Eumitotic. *Ascaris*.
 - (b) entire chromosomes lying opposite one another. Pseudomitotic. *Cyclops*.
 2. 'Konjugationstypus'; chromosomes not in the form of tetrads but are formed by the conjugation of two chromosomes. Vertebrates and plants.

The Schreiners (1908), Grégoire (1909), and Wassermann (1913), however, all maintain that a 'Primärtypus' does not exist after studying *Zoogonus mirus*, the very form for which Goldschmidt proposed this behaviour. Later workers have failed to observe this scheme of maturation in species other than *Zoogonus*, and Goldschmidt, himself (1908), abandoned the idea in his study of *Dicrocoelium lanceolatum*. Gametogenesis in *Cryptocotyle lingua* also negates the existence of a 'Primärtypus' since, in this form, pseudoreduction occurs before the first maturation division and there is good reason to think that parallel conjugation or parasynapsis takes place. The only evidence of bivalency of the chromosomes (prior to their division) is their larger size. They exhibit no lines of fusion that can be distinguished.

In the above description of oogenesis, it has been assumed

that the first maturation division is reductional and the second equational, or in other words, that *Cryptocotyle* follows the hetero-homotypic scheme of maturation. Some reason for this assumption must be given. The chief one is the almost identical resemblance of the first maturation division of the oocyte to the reduction division of the spermatocyte. The chromosomes possess the same general characteristics. Their manner of longitudinal division is much the same in both cases. The chromosomes of the second polar spindle appear slender and filamentous when compared with those of the reduction division in the spermatocytes. It is of general occurrence that the first maturation division is reductional and it is believed, for reasons already given, that *Cryptocotyle lingua* is no exception to this rule.

Many workers have debated the significance of the karyosome and its importance in cell behaviour. This structure has not been closely observed for the purpose of determining its relation to the amount of chromatin in the germ-cells. In mature worms, however, it stains faintly and exhibits a clear centre with an outer reticular appearance, while the chromatin granules stain intensely. In immature worms, the karyosome takes a deep stain and appears homogeneous in structure, while the chromatin, whether dispersed or condensed in heavy threads, stains very faintly. This would indicate that in either case there is a marked difference in the composition of the karyosome and the chromatin. The karyosome has been of use in identifying certain stages, viz. the primary spermatogonia and the oogonia. These usually possess two such structures of about equal size, while their later stages have only one or a large and a small one.

Cytoplasmic inclusions have received no consideration in this work. Their observation requires special technique which was not employed since the nucleus and its constituents were the objectives of the present study. Most of the workers have been unsuccessful in observing mitochondrial inclusions or other formed elements in the cytoplasm of the trematode germ-cells. The cytoplasm is much reduced in these cells and the fact that it is apparently left behind in the case of the spermatozoa would

seem to indicate that the nucleus and its behaviour are of most importance in the germ-cell.

No degenerating cells or granules that might be interpreted as nutritive material have been observed in either the testes or the ovary of *Cryptocotyle lingua*. This species, like all other trematodes, possesses a loose histological organization with the body fluid permeating the intercellular spaces of the mesenchymatous tissue. Cell-boundaries are indistinct, even in the gonads. For these reasons, the writer is inclined to believe with Schellenberg that the germ-cells are nourished while in the gonads by the body fluid and not by degeneration of the cells of the germinal line. After the oocytes leave the ovary, however, what appear to be degenerating cells have been observed in their cytoplasm. These, as stated above, may possibly be either vitelline cells or oocytes.

It is the opinion of many investigators that the hermaphroditic trematodes have the facultative power of self-fertilization, although copulation is characteristic. Stunkard (personal communication) reports having seen *Cryptocotyle* with the copulatory organ inserted in its own vaginal opening, pouring large numbers of sperms into its own seminal receptacle.

VI. SUMMARY.

1. The chromosomes of *Cryptocotyle lingua* have been counted and their shapes and sizes determined from observations on somatic and germinal cell divisions.

2. The differences in structure and staining reactions of the germinal cells in mature and immature worms are described.

3. Oogenesis and spermatogenesis have been followed from the primary germ-cells of the immature worm to the early cleavage stages.

4. More evidence has been added to that indicating the non-existence of the 'Primärtypus' of maturation postulated by Goldschmidt.

5. Gametogenesis in *Cryptocotyle* has been compared with that in other species.

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DESCRIPTION OF PLATES 26-31.

LETTERING.

b, bouquet; *dspc*₂, division of the secondary spermatocyte; *k*, karyosome; *pb*₁, first polar body; *pb*₂, second polar body; *pn*, pronucleus; *rd*, reduction division; *sh*, shell; *sp*, sperm; *spc*₁, primary spermatocyte; *spg*₁, primary spermatogonia; *spg*₂, secondary spermatogonia; *spg*₃, tertiary spermatogonia; *spt*, spermatid; *yk*, yolk; *ykg*, yolk globule.

PLATE 26.

Fig. 1.—Anaphase of a somatic mitosis.

Fig. 2.—The chromosomes arranged in pairs.

Fig. 3.—Prophase of a somatic mitosis.

Figs. 4 and 5.—Consecutive sections containing the prophase of a somatic mitosis.

Fig. 6.—Section of a rather mature testis.

Fig. 7.—Resting primary spermatogonium.

Fig. 8.—Primary spermatogonium, spireme stage.

Fig. 9, *a* and *b*.—Consecutive sections of primary spermatogonial division (prophase).

Fig. 10.—Secondary spermatogonia.

Fig. 11, *a* and *b*.—Consecutive sections of a dividing secondary spermatogonium.

Fig. 12.—A cluster of tertiary spermatogonia.

Fig. 13.—Dividing tertiary spermatogonia.

PLATE 27.

Fig. 14.—Section through a cluster of primary spermatocytes.

Fig. 15, *a* and *b*.—Two of eight bouquet stages observed in a cluster of primary spermatocytes.

Fig. 16.—Section showing three of the eight groups of bivalent chromosomes before the reduction division of the primary spermatocyte.

Figs. 17, 18, and 19.—Various stages of the reduction division, showing the longitudinal separation of the components of the bivalent chromosomes.

Fig. 20.—A cluster of secondary spermatocytes in the resting stage.

Fig. 21, *a*, *b*, and *c*.—Condensation figures of spermatocyte nuclei.

Fig. 22.—A group of dividing secondary spermatocytes.

Fig. 23.—Young spermatids.

Fig. 24.—Spermatids beginning to elongate.

Fig. 25.—Later stage of elongation of the spermatids.

PLATE 28.

Figs. 26 and 27.—Further stages of spermatid elongation.

Fig. 28.—Stage showing the orientation of the spermatids.

Fig. 29.—Resting oocyte.

Figs. 30, 31, 32, and 33.—Stages leading to syndesis in the oocyte.

Fig. 34.—Bouquet stage of the oocyte.

Figs. 35, 36, and 37.—Oocytes with nutritive cells (nuclei). These were found in the ootype.

PLATE 29.

Fig. 38.—Resting oocyte as it appears just before sperm penetration.

Fig. 39.—An unusual case in which the nucleus is small and the chromatin is much condensed. The sperm appears to lie at or near the surface of the oocyte and the shell is not yet formed.

Fig. 40.—End-view of an egg showing the oocyte and sperm.

Figs. 41 and 42.—Condensation stages of oocyte nuclei.

Figs. 43 and 44.—Stage showing the bivalent chromosomes of the first maturation division. The sperm is much shortened and thickened.

Fig. 45.—Stage interpreted as the fertilization spindle.

Fig. 46, *a* and *b*.—Consecutive sections of reduction division anaphase.

Fig. 47.—The pronuclei with the second polar body.

PLATE 30.

Figs. 48 and 49.—Sections of rather immature testes showing various stages of spermatogenesis (photomicrograph).

PLATE 31.

Fig. 50.—Section of a rather mature testis (photomicrograph).

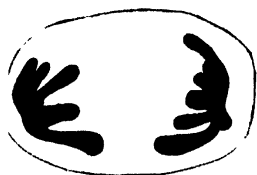
Fig. 51.—Prophase of oocyte reduction division showing the six bivalent chromosomes and condensed sperm (photomicrograph).

Fig. 52.—Section of a rather mature ovary (photomicrograph).

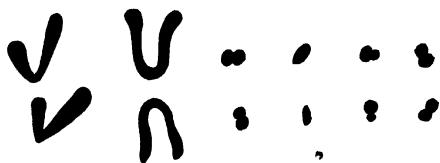
Fig. 53.—Second maturation division showing the granular appearance of the sperm and the filamentous nature of the chromosomes of this division (photomicrograph).

Fig. 54.—The same.

Fig. 55.—Two-cell cleavage stage (photomicrograph).



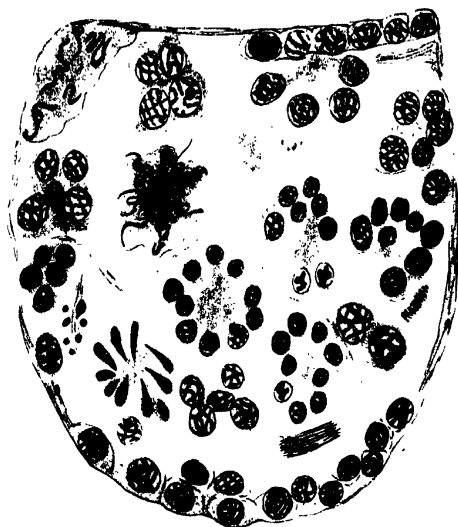
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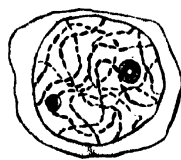
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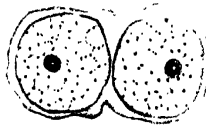
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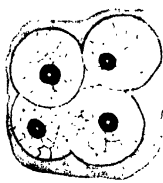
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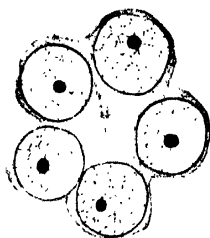
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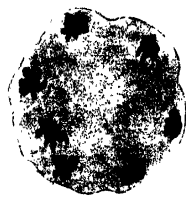


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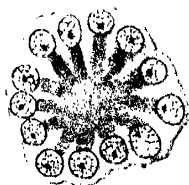
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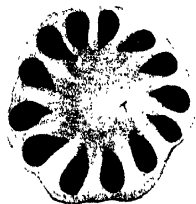
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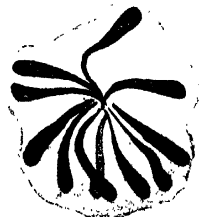
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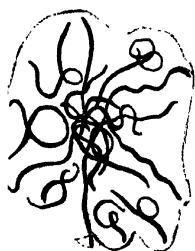
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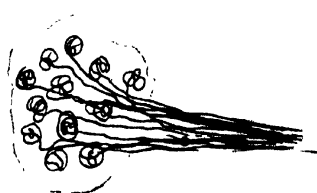
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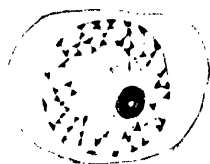
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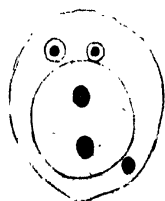
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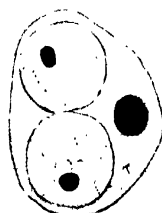
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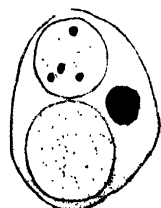
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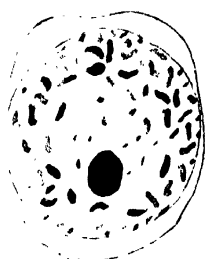
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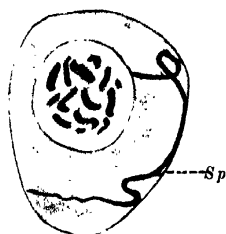
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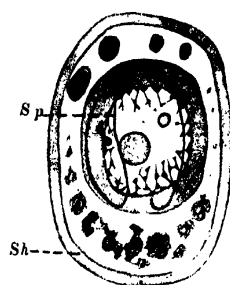
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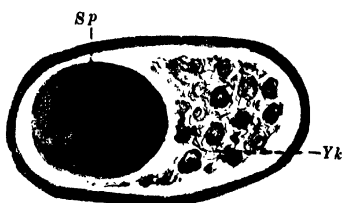
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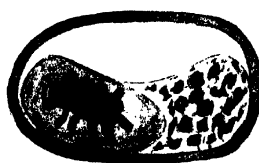
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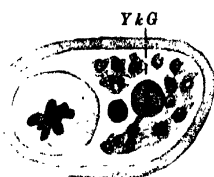
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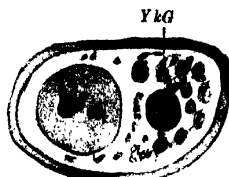
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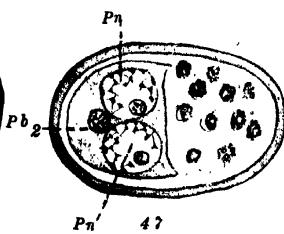
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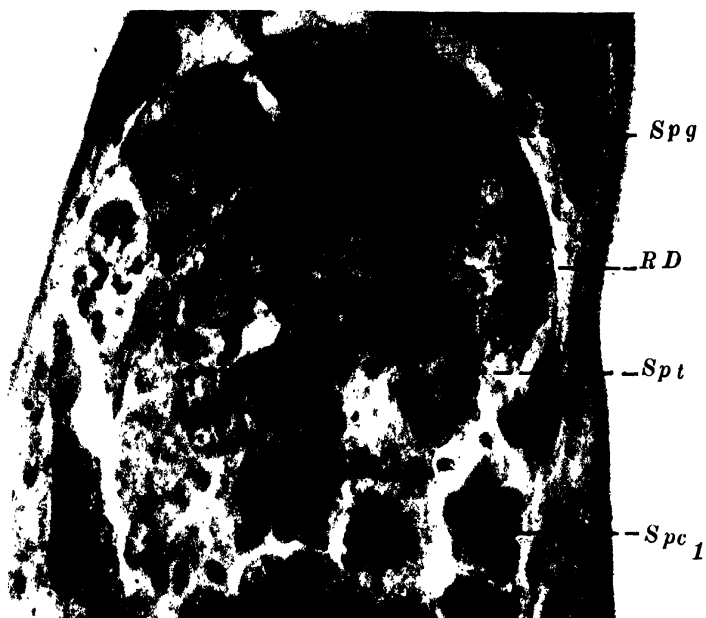
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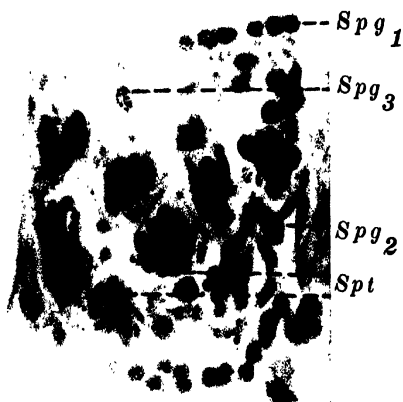


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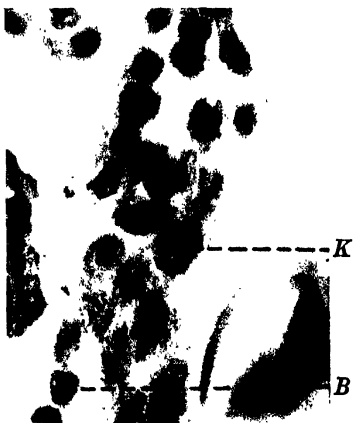




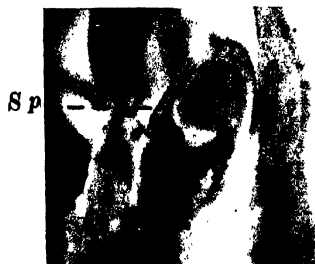
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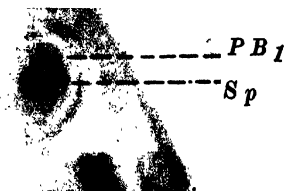
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The Development of the Skull of *Scyllium (Scyliorhinus) canicula* L.

By

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With Plates 32-7 and 27 Text-figures.

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INTRODUCTION.

It is little short of remarkable that the skull of *Scyllium canicula*, one of the commonest types used for laboratory dissection, should not have been the object of more detailed and recent study than it has. Parker's (1878) famous paper was excellent, although it contained a few mistaken observations, as pointed out by Ridewood (1895 and 1897), and it remains the sole complete work on the development of the skull of *Scyllium*. Some valuable observations on certain features of the development are contained in papers by van Wijhe (1904) and by Goodrich (1918); the former being principally concerned with *Squalus acanthias*, and the latter with the segmentation of the head. On the other hand, the skull of *Squalus acanthias* has been well and thoroughly studied by Sewertzoff (1899), Wells (1917), van Wijhe (1922), and Mori (1924). It shows certain differences as compared with *Scyllium*, and

this paper is presented as an attempt to fill in a gap in our knowledge and to provide material for a detailed comparison between the developments of two fairly closely related forms. In addition, a discussion of the results obtained will lead to a consideration of certain problems of general interest in vertebrate craniogeny.

The material on which the present study is based consisted of some thirty embryos of *Scyllium canicula* prepared according to van Wijhe's method (1902 and 1922) for demonstrating cartilage. After staining and while being dehydrated, the embryos were dissected under the binocular microscope with the help of very fine Swiss watch-maker's forceps, and mounted in glass-cells which allow of study under the compound microscope on all sides. In addition, the whole of Professor Goodrich's and the late Dr. Jenkinson's splendid collection of sections was available for the purpose of checking the results obtained by a study of the whole preparations, and for determining the relations of those structures which are invisible by the van Wijhe technique. The figures which illustrate this paper were drawn under the camera lucida at a magnification of 25 diameters, and the author is indebted to his wife for assistance in preparing them for reproduction. A carefully selected set of transverse sections is given in the form of text-figures, for the purpose of elucidating certain matters. The work was carried out in the Department of Zoology and Comparative Anatomy of the Oxford University Museum, and, as always, the author enjoyed the kind encouragement of Professor E. S. Goodrich, F.R.S., to whom he wishes to express his gratitude.

Before the work had progressed very far, it had become obvious that embryos of a given length from Naples were not identical with those of the same length from Plymouth. On the whole, the Naples material showed a tendency to delay in the chondrification of the anterior and dorsal regions of the skull, as compared with Plymouth material of the same degree of development. The difference is purely an embryological and not a morphological one, for the structures in the Naples embryos, when they do chondrify, exhibit relations identical with those shown by the Plymouth embryos. In addition to

Table of material of *Scyllium canicula* used for this work.

<i>Stage.</i>	<i>Length.</i>	<i>Provenance.</i>	<i>Illustrated in</i>
	mm.		
1	24	Plymouth	Fig. 1, Pl. 32
2	25	?	Fig. 2, Pl. 32
3	28	Naples	Fig. 3, Pl. 32
4	29	Naples	Fig. 4, Pl. 32
5	29½	Naples	Figs. 5, 6, Pl. 32
6	30	Naples	Figs. 7, 8, Pl. 32
7	30	Plymouth	Figs. 9, 10, Pl. 33, and Text-figs. 1, 2
8	34	Plymouth	Figs. 11, 12, Pl. 33
9	35	Plymouth	Figs. 13, 14, Pl. 34
10	36	Plymouth	Figs. 15, 16, Pl. 34; fig. 17, Pl. 35
11	36	Naples	Fig. 18, Pl. 35
12	37	Plymouth	Figs. 19, 20, Pl. 36
13	45	Naples	Figs. 21, 22, 23, 24, Pl. 37
Adult	—	Plymouth	Figs. 26, 27, 28, Pl. 36, and 25, Pl. 37.

the material enumerated in the table given above, several Naples embryos were studied and drawn, but they are not reproduced in the paper, for they merely show imperfection or retardation of chondrification. The matter, however, is interesting, as revealing a variation in the time factor in development within a species. Analogous variations with regard to *Squalus acanthias* were found by van Wijhe (1922); he observed that cartilage made its first appearance in embryos 22 mm. long from Heligoland and Helder, but 32 mm. long from Boston; further, embryos collected in summer from Heligoland were more advanced in their development than embryos of similar size collected in winter from Helder.

The author wishes to express his appreciation of the kind help given him in provision of material by the Plymouth Laboratory of the Marine Biological Association, by the Stazione Zoologica at Naples, and by Professor J. P. Hill, F.R.S.

DESCRIPTION OF STAGES.

Stage 1 (24 mm., Plymouth, fig. 1, Pl. 32).

In several embryos of this stage of development, it has been possible to see that the parachordal cartilages are the first

elements of the skull to become chondrified. They extend from a point level with the front wall of the auditory sac to a point above the first gill-slit, and are slightly thicker behind than they are in front. There is as yet no sign of the dorsal process or of the occipital arch. Goodrich (1918) has shown that the sheet of mesenchyme from which the parachordal arises is doubtless formed from sclerotomes 4 and 3, and perhaps also 2 and 1, though no signs of segmentation are any longer visible. On the other hand, scleromeres are formed in segments 5, 6, and 7, as condensations of mesenchyme. At later stages, the cartilage which arises from these condensations becomes fused with the parachordal plate forming its occipital region, while the condyles appear to be derived from the scleromere of segment 8.

Stage 2 (25 mm., fig. 2, Pl. 32).

At this stage a number of other structures have made their appearance, and are present as very young cartilage. These are: the skeletal elements of the mandibular and hyoid arches (palato-quadrates and Meckel's cartilage; hyomandibula and ceratohyal); the trabeculae, and the orbital cartilages. All these structures are independent. Meckel's cartilage appears to possess only one centre of chondrification, and not two (on each side) as reported by van Wijhe (1922) in *Squalus*. As Goodrich showed, the angle made between the parachordal and the trabecula is not as acute in *Scyllium* as it is in *Squalus*.

Stage 3 (28 mm., Naples, fig. 3, Pl. 32).

The parachordal cartilages are, of course, situated immediately beneath and median to the auditory sacs, and at this stage they can be seen to send a process outwards and forwards on each side towards the under surface of the front of each sac. At the same time, the lateral edge of the parachordal is no longer straight. Just behind the process described above, and which is really the rudiment of the anterior basicapsular commissure, the edge of the parachordal forms the so-called lamina basiotica, and bears an uprising knob, which is the rudiment of the dorsal process. Behind this, the parachordal is narrower than in the region of the lamina basiotica.

Stage 4 (29 mm., Naples, fig. 4, Pl. 32).

The cartilage of the parachordal is now continuous with a thin sheet of cartilage which underlies the front region of the auditory sac. In *Scyllium*, therefore, as Goodrich has already shown (1918), the auditory capsule chondrifies from the first in continuity with the parachordal, whereas in *Squalus* van Wijhe showed that the auditory capsule had independent centres of chondrification. Van Wijhe is of the opinion that the early and close association between the parachordals and the auditory capsule indicates that the origin of the brain-case was associated with the provision of a firm attachment for the ear as an organ of balance. The conditions in *Scyllium* therefore give him even stronger support than do those of *Squalus*.

It may be noticed that the cartilage cells of the parachordals extend forwards a little way along the notochord sheath (without yet invading it), but the foremost ends of the parachordals diverge from one another and from the notochord, which extends forwards freely between them.

This embryo is of interest for two further reasons. It shows the earliest origin of the vertebral elements in the form of the basidorsal cartilages, and they are entirely independent and distinct from one another. This is important in view of the fact that van Wijhe (1922) has described the vertebral elements as arising from four continuous bands of cartilage along the notochord, the dorsal bands corresponding to the basidorsals and the ventral bands to the basiventrals, in both *Scyllium* and *Squalus*. At later stages, he shows in *Squalus* that the ventral and dorsal band fuse on each side into a single band, and that eventually the separate basidorsal and basiventral cartilages are separated off from one another, presumably, as he thinks, as a result of the growth in length and diameter of the notochord and of its sheath. My studies of *Scyllium* have shown me nothing of this, and the basidorsal and basiventral cartilages are independent from the start, as they appeared to me to be in *Heterodontus* (de Beer, 1924). It is only at subsequent stages that adjacent basidorsals and basiventrals become connected by a thin film of cartilage along the outer surface of the elastica externa. There appears here,

therefore, to be a conflict of evidence concerning the method of origin of the vertebral column, which I am at a loss to understand. Attention may be directed to the fact that in the anterior region of the vertebral column, the basidorsals appear before the basiventrals, as is also the case in *Salmo* (de Beer, 1927).

The other point of interest with regard to this embryo concerns the polar cartilage. As is now well known, van Wijhe discovered a polar cartilage in *Squalus* (1904) between the trabecula and parachordal, and the independent existence of this structure has been confirmed in other forms. In *Squalus* the polar cartilage first becomes attached to the hind end of the trabecula of its own side, and then with the under surface of the front edge of the parachordal. After numerous fruitless attempts to find the polar cartilages as independent structures in *Scyllium* (a quest in which Professor van Wijhe was also unsuccessful, as he kindly informed the author) it would seem that they are at last discovered in this embryo, not as independent chondrifications, but as nodules of cartilage attached to the under surface of the front edge of the parachordals, free from the trabeculae, and sending a small sharp process towards one another. As will be shown in connexion with later stages, these structures answer all the requirements of polar cartilages, but the new feature which they show is that they are more closely associated with the parachordals than with the trabeculae. This is, after all, a difference of small importance, and in *Amia* (Pehrson, 1922; de Beer, 1926) the polar cartilages would seem to be in a condition intermediate between that of *Squalus* and of *Scyllium*, for in *Amia* the polar cartilage arises in association with both the trabecula and parachordal. The condition in *Scyllium* is, however, interesting as suggesting the possibility that the cartilage which appears to form the anterior region of the parachordal may in reality represent the polar cartilage (as, for instance, in Teleostei).

Stage 5 (29½ mm., Naples, figs. 5, 6, Pl. 32).

This embryo agrees with the previous one in that the polar cartilages are visible, attached to the under surface of the front

end of the parachordals. The trabeculae and orbital cartilages are still free. The parachordals now show a prominence at their hind ends, forming the rudiment of the occipital arch. It may also be noticed for comparison with earlier stages that the hind end of the parachordals now lies over the second gill-slit. In the vertebral column, the neural arches of the first three vertebrae have appeared, as minute nodules of cartilage, separate and independent from the basidorsals. As regards the visceral arch skeleton, ceratobranchials and epibranchials are now present in the first, second, and third branchial arches. They arise in the form of a U wrapped round the adductor muscles, and when the arms of the U meet, the characteristic foramina are formed.

Stage 6 (30 mm., Naples, figs. 7, 8, Pl. 32).

The trabeculae are attached to the polar cartilages, so that now for the first time the floor of the brain-case is continuous. There is, however, no continuity between right and left sides, for the notochord completely separates the parachordals. The orbital cartilage is now attached by the pila antotica to the upper surface of the front edge of the parachordal of its own side. In *Squalus* van Wijhe describes an independent chondrification for the cartilago antotica (pila antotica) as well as for the cartilago supraorbitalis (orbital cartilage). In *Scyllium* a separate origin for the pila antotica has not been found. The lateral surface of the auditory capsule is now chondrified, but it has not been possible to make out the two centres of chondrification discovered by van Wijhe in *Squalus*. The anterior end of the auditory capsule is attached to the parachordal by means of the anterior basicapsular commissure, but its posterior end is free. Between the capsule and the lateral edge of the parachordal there is therefore a slit, which, in its anterior region represents a basicapsular fenestra, such as is present in *Salmo*. But owing to the absence of a posterior basicapsular commissure, this fenestra is confluent with the fissura metotica behind it. The dorsal process of the parachordal marks the approximate line of separation between these two portions of the slit. The glossopharyngeal nerve passes out behind the dorsal process and beneath the auditory capsule, in

the *fissura metotica*. The occipital arch is pierced by a foramen for the posterior cranial root of the hypoglossal nerve. Van Wijhe (1904) imagined that this condition, which he also observed, indicated that the occipital arch represented a single neural arch, pierced by a ventral nerve-root foramen, as are the neural arches of *Squalus*, but not of *Scyllium*. Goodrich (1918), on the other hand, has shown that it is much more probable that the occipital arch represents two arches, related to the seventh and sixth sclerotomes respectively, and enclosing a hypoglossal root between them. The conditions in *Scyllium* lend strong support to Goodrich's view, for in some cases, the enclosure of the nerve-root in a foramen is incomplete, and it then lies in a groove between the large posterior occipital arch and the smaller anterior arch. There are, therefore, definite traces of metameric segmentation in the metotic region of the skull of *Scyllium*. In the visceral arch skeleton, the pharyngobranchials have now appeared in the first to third branchial arches. Basiventral cartilages are now present in the vertebral column.

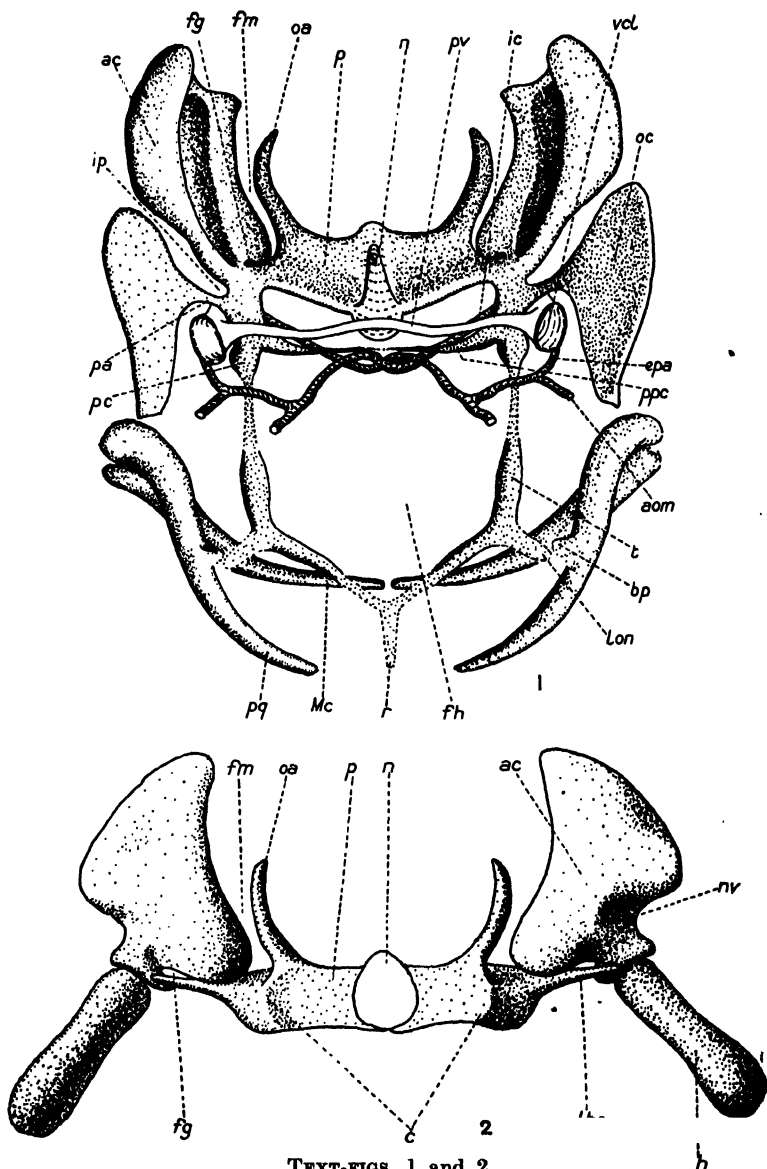
Stage 7 (30 mm., Plymouth, figs. 9, 10, Pl. 33, and Text-figs. 1, 2).

This is one of the most interesting and important of the early stages of development of the skull of *Scyllium*. Anteriorly, the trabeculae converge towards an independent median rostral cartilage, while, on each side, each trabecula bears a process directed outwards and forwards; the ethmoid process of Sewertzoff (1899), or the lamina orbitonasalis of van Wijhe. In front of the rostral cartilage on each side the paired nasal cartilages have appeared, in the form of transverse bars each bearing three backwardly projecting processes, the outermost two meeting round the external nostril and enclosing it in a ring. In front of these nasal cartilages is another pair of independent cartilages, representing the rudiments of the front wall of the nasal capsule.

Although the polar cartilage is attached to trabecula and parachordal, its position is clearly marked out by notches: one in front, representing the original separation between the polar

cartilage and the trabecula, and lodging the efferent pseudo-branchial artery; and one behind, between the polar cartilage and the antero-ventral edge of the pila antotica, and lodging the pituitary vein. The polar cartilages were seen at an earlier stage to have a process directed towards their fellow of the opposite side. In this embryo, these processes have become interconnected by means of a transverse cartilage which will here be called the postpituitary commissure. As the reconstruction given in Text-fig. 1 shows, this commissure lies behind the point of entry of the internal carotid arteries and beneath the notochord. It is therefore not an acrochordal, and it is not the same as the cartilage which van Wijhe found in *Squalus* and which lies in front of the point of entry of the carotids. The latter, or precarotid commissure, is present in later stages of *Scyllium*, as will be seen below.

The orbital cartilage has extended and lapped round the trochlear nerve, which is therefore enclosed in a foramen. From the postero-dorsal corner of the orbital cartilage, a process is directed backwards towards the auditory capsule; this is the rudiment of the taenia marginalis, which will eventually convert the incisura prootica into the foramen prooticum. The auditory capsule is now fairly well developed, and the three canals and the septa separating them from the main cavity of the capsule can be seen by transparency. Anteriorly, the capsule is, of course, attached to the parachordal by the broad anterior basiocapsular commissure; posteriorly it is still free. The embryo reconstructed in Text-figs. 1 and 2 is slightly further developed than that illustrated in figs. 9 and 10, Pl. 93, and the posterior view shows that a shelf of cartilage stretches out from the parachordal on each side, beneath the posterior portion of the auditory capsule. This cartilaginous shelf plays an important part in producing the apparent passage of the glossopharyngeal nerve through the auditory capsule, for in this region the auditory capsule has no cartilaginous floor of its own, and the nerve, lying above the shelf, passes through what is really a tunnel of which the upper wall has broken down. Should a name be required for this shelf, lamina hypotica may be suggested, so as to show that it is morphologically ventral to the floor of the



TEXT-FIGS. 1 and 2.

Reconstruction from serial sections of an embryo of *Scyllium canicula* about 30 mm. long, seen from in front.

Reconstruction from serial sections of an embryo of *Scyllium canicula* about 30 mm. long, seen from behind.

auditory capsule, and in order to distinguish it from that other portion of the parachordal plate which van Wijhe has called the

EXPLANATION OF LETTERING.

a, articular facet for the hyomandibula; *abc*, anterior basicapsular commissure; *ac*, auditory capsule; *an*, abducens nerve; *aom*, arteria ophthalmica magna; *as*, auditory sac; *asc*, anterior semicircular canal; *aun*, auditory nerve; *ba 1*, branchial arch 1 (to 5); *bc*, basicochlear fissure; *bcf*, basicapsular fenestra; *bd 1*, basidorsal 1 (to 5); *bh*, basihyal; *bp*, basal process; *br 1*, branchial rays of first (to fifth) arch; *bv 1*, basiventral 1 (to 7); *bvc*, basivestibular commissure; *c*, occipital condyle; *cac*, cavity of auditory capsule; *cb 1*, ceratobranchial of first (to fifth) arch; *ce 1*, centrum of first vertebra; *cf*, foramen for internal carotid artery; *ch*, ceratohyal; *cn*, cartilage connecting front and side walls of nasal capsule; *csp*, cartilage connecting palatoquadrate and lamina orbito-nasalis; *dcs*, ductus canalis semicircularis posterioris; *del*, ductus endolymphaticus; *dm*, dura mater; *dp*, dorsal process of parachordal; *ds*, dorsum sellae; *e*, eye; *eb 1*, epibranchial of first (to fifth) arch; *ee*, elastica externa; *eha*, efferent hyoidean artery; *ei*, elastica interna; *en*, nostril; *epa*, efferent pseudobranchial artery; *ead 1*, dorsal extrabranchial of first (to fourth) arch; *exv 1*, ventral extrabranchial of first (to third) arch; *f*, foramen magnum; *fa*, foramen for auditory nerve; *fb*, basicranial fenestra; *fel*, foramen for ductus endolymphaticus; *fep*, foramen for efferent pseudobranchial artery; *fg*, foramen for glossopharyngeal nerve; *fh*, hypophysial fenestra; *fic*, interorbital canal for pituitary vein; *fl*, foramen enclosing lateral-line canal; *flt*, foramina for twigs of superficial ophthalmic branch of facial nerve; *fm*, fissura metotica; *fn*, facial nerve; *fnc*, front wall of nasal capsule; *fo*, foramen for optic nerve; *foa*, foramen for orbital artery; *foc*, foramen for oculomotor nerve; *fol*, olfactory foramen; *fom*, orbito nasal foramen; *fp*, palatine branch of facial nerve; *fpr*, foramen prooticum; *fsf*, foramen for superficial ophthalmic branch of facial nerve; *fso*, superficial ophthalmic branch of facial nerve; *fst*, foramen for superficial ophthalmic branch of trigeminal nerve; *ft*, foramen for trochlear nerve; *fv*, foramen for vein; *g*, gap between lamina hypotica and true floor of auditory capsule; *gc*, canal for passage of glossopharyngeal nerve; *gn*, glossopharyngeal nerve; *gs 1*, gill-slit 1 (to 5); *h*, hyomandibula; *ha*, hyoid arch; *hb*, hindbrain; *hbr 1*, hypobranchial of the first (to third) arch; *hf*, foramen (or foramina) for hypoglossal nerve; *hn*, hypoglossal nerve; *hrd*, dorsal group of hyal rays; *hrv*, ventral group of hyal rays; *hy*, hypophysis; *i 1*, interdorsal 1 (to 5); *ic*, internal carotid artery; *iom*, inferior oblique muscle; *ip*, incisura prootica; *is*, invaded sheath of notochord; *l*, lagena; *lb*, limit between planum antorbitale and preoptic root of orbital cartilage; *lbp*, lamina basiotica; *lc*, labial cartilage; *lho*, lamina hypotica; *llc*, lateral-line canal; *lnc*, side wall of nasal capsule; *lon*, lamina orbito-nasalis; *lsc*, lateral semicircular canal; *ma*, mandibular arch; *maa*, macula ampullaris anterioris; *mal*, macula ampullaris

lamina basiotica, and which forms the floor of the more anterior part of the capsule (1922, p. 281). The roof of the auditory capsule is incomplete, and through a hole it is possible to see the basicapsular fenestra. Eventually this fenestra will be obliterated when the lateral edge of the parachordal (lamina basiotica) and the ventral edge of the wall of the auditory capsule have joined.

The hypoglossal nerve on one side of the embryo illustrated in figs. 9 and 10, Pl. 33, has become enclosed in a foramen at the base of the occipital arch, but on the other side it still lies in a notch, between the anterior and posterior occipital arches, as described by Goodrich.

lateralis; *man*, macula neglecta; *map*, macula ampullaris posterioris; *mas*, macula sacculi; *Mc*, Meckel's cartilage; *mnc*, median wall of nasal capsule; *mru*, macula recessus utricularis; *ms*, mandibular visceral cleft; *n*, notochord; *na 1*, neural arch of first (to third) vertebra; *nc*, nasal cartilage; *nci*, inner process of nasal cartilage; *ncm*, middle process of nasal cartilage; *nco*, outer process of nasal cartilage; *nep*, notch for efferent pseudobranchial artery; *non*, notch for optic nerve; *npv*, notch for pituitary vein; *ns*, nostril; *nt*, spinal cord; *nv*, notch for vena capitis lateralis; *nve*, notch for vein; *oa*, occipital arch; *oa 1, 2, or 3*, segmental occipital arches; *oc*, orbital cartilage; *ofm*, obliterated region of fissura metotica; *on*, oculomotor nerve; *opn*, optic nerve; *ora*, orbital artery; *os*, orbital sinus; *p*, parachordal; *pa*, pila antotica; *pb*, pituitary body; *pbc*, posterior basicapsular commissure; *pc*, polar cartilage; *pcc*, precarotid commissure; *pch*, perichordal commissure; *pcv*, posterior canal vacuity; *pev*, plica encephali ventralis; *pf*, parietal fossa; *pl*, planum antorbitale; *pma*, premandibular arch; *pn*, profundus nerve; *ppb*, peripharyngeal band; *ppc*, postpituitary commissure; *pq*, palato-quadrate; *pr 1*, pharyngobranchial of the first (to fifth) arch; *prr*, preoptic root of the orbital cartilage; *psc*, posterior semicircular canal; *pv*, pituitary vein; *r*, rostrum; *rem*, rectus externus muscle; *rif*, rectus inferior muscle; *rim*, rectus internus muscle; *rm*, rectus superior muscle; *ru*, recessus utricularis; *s*, sacculus; *sbo*, subocular cartilage; *sc*, spiracular cartilage; *sl*, lateral rostral process; *sm*, median rostral process; *sp*, spiracular pouch or cleft; *spo*, supraorbital cartilage; *spc*, subpituitary space; *srp*, suprarostal process; *sso*, spiracular sense-organ; *st*, stomodæum; *stp*, stomodæal pouch; *sv 2*, second spinal ventral nerve-root; *t*, trabecula; *tep*, tectum posterior; *tf*, foramen for thyroid gland-stalk; *th*, rudiments of thymus gland; *tm*, taenia marginalis; *tn*, trigeminal nerve; *tp*, trabecular plate; *ts*, tectum synoticum; *tso*, superficial ophthalmic branch of trigeminal nerve; *u*, utricle; *uc*, unpaired cartilage representing hypobranchials of fourth and fifth arches; *v*, velum; *vcl*, vena capitis lateralis; *vn*, vagus nerve; *vs*, ventral sac of hypophysis.

At this stage the notochord sheath has begun to be invaded by the cartilage cells of the parachordals. The result of this invasion, which begins at the posterior end of the parachordals, is to establish cartilaginous connexion between the parachordals of the two sides, above and beneath the notochord, forming what may be called a perichordal commissure.

In the vertebral column, it is noticeable that the neural arches of the first and second vertebrae are situated considerably in front of their respective basidorsals, while the neural arches and basidorsals of the third and following vertebrae are in contact. At this stage the interdorsal cartilages have appeared, as independent cartilages, between the neural arches and on the same level as them.

The palato-quadrates show a thickening on its dorsal surface near the front end, which foreshadows the basal (orbital) process. Further back, the fifth branchial arch has now chondrified.

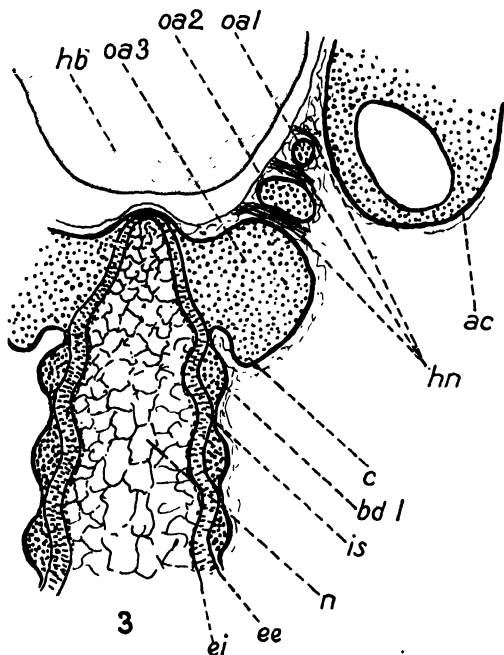
Stage 8 (34 mm., Plymouth, figs. 11, 12, Pl. 33).

This stage follows on easily from the previous one. The trabeculae have fused with the median rostral cartilage, and in so doing have enclosed the hypophyseal fenestra from in front. In the hind region of this fenestra, the postpituitary commissure has appeared as an independent transverse bar of cartilage, between the polar cartilages, beneath the notochord, and behind the point of entry of the carotid arteries. Thus, while the postpituitary commissure forms a posterior boundary to the hypophyseal fenestra, it does not form the anterior boundary of the basicranial fenestra, for it is ventral to the level of that fenestra. In other words, the postpituitary commissure is not the same thing as the acrochordal.

The cartilages representing the rudiments of the front wall of the nasal capsule have now become attached to the rostrum, while in the nasal cartilages the outermost two prongs are no longer fused behind the nostril. The lamina orbitonasalis is further developed than in previous stages, and the taenia marginalis now connects the orbital cartilage with the auditory capsule. The latter still shows a basicapsular fenestra, visible through a hole in the roof. The hinder part of the auditory capsule is now underlain by the lamina hypotica, extending

sideways like a shelf from the hindmost part of the parachordals. The glossopharyngeal nerve emerges from a notch between the lamina hypotica and the auditory capsule.

There are now two hypoglossal nerve-roots enclosed in foramina on each side, and a horizontal section through this region

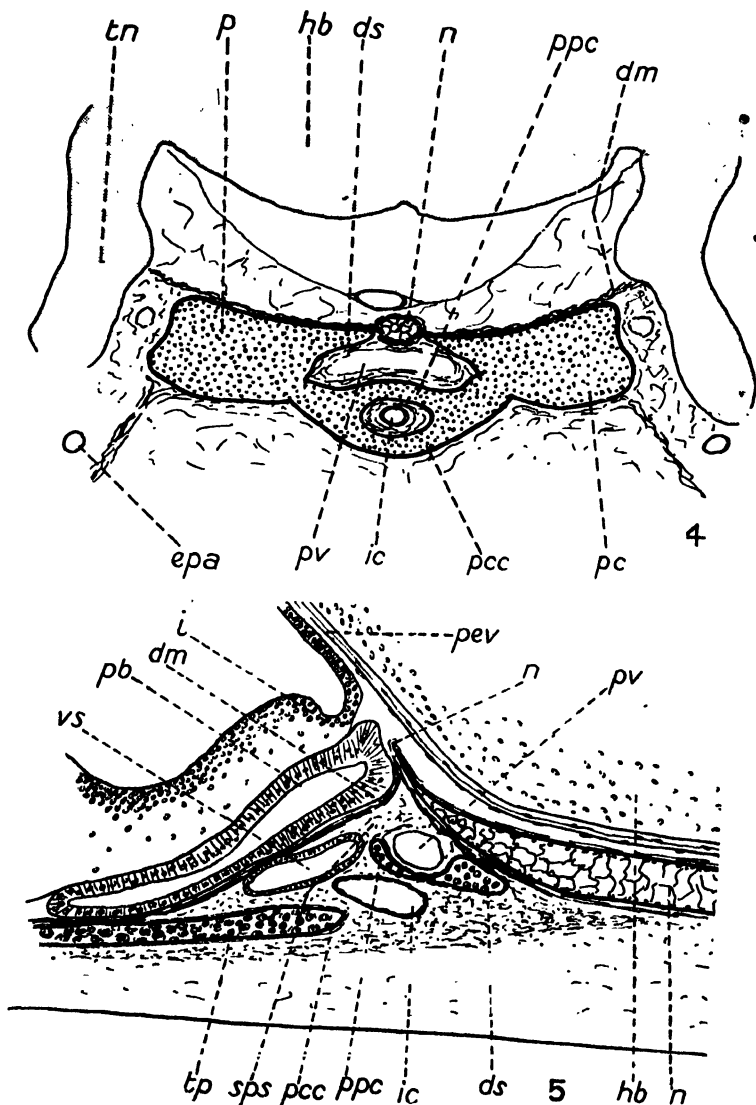


TEXT-FIG. 3.

Horizontal section through the occipital region of an embryo of *Scyllium canicula* 32 mm. long (32 H, 9-1-4), showing the metameric segmentation of the occipital arches in the posterior parachordal region.

(Text-fig. 3) is very instructive as to the manner in which these nerve-roots are enclosed between what are really segmentally repeated occipital arches. The perichordal commissure has extended farther forwards, and, in the visceral skeleton the dorsal extrabranchial cartilages have appeared.

The basal process on the palato-quadrate is a well-developed



TEXT-FIGS. 4 and 5.

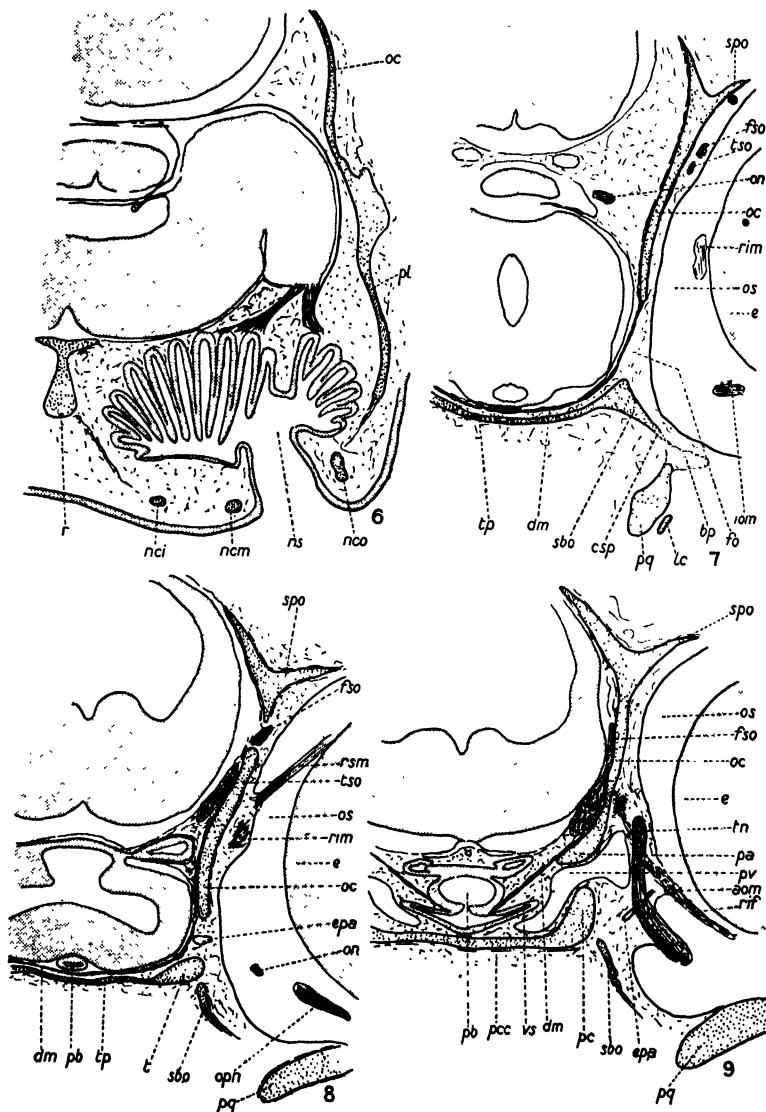
Transverse section (G. R. de B. 29-5-3-6) showing the precarotid commissure, postpituitary commissure, and dorsum sellae, all distinct. Median longitudinal section (J. H. J., young, 18-1-3) showing subpituitary space, trabecular plate, precarotid commissure, postpituitary commissure, dorsum sellae, internal carotid arteries, pituitary vein.

knob, projecting upwards and outwards, beneath the lamina orbitonasalis. The relations of this region are of considerable interest, for, on the one hand, Edgeworth has observed a cartilaginous connexion between the palato-quadrato and the lamina orbitonasalis (antorbital process; 1925, p. 255), and on the other, Sewertzoff and Disler (1924) have reported the existence of a (mesenchymatous?) separate pharyngomandibular element (in *Scyllium*, *Squalus*, and *Mustelus*), which ultimately becomes attached to the basal (orbital) process of the palato-quadrato. I have observed the mesenchymatous mass to which Sewertzoff and Disler have applied the term pharyngomandibular, but its independence appears to be doubtful. It seems to be a mesenchymatous connexion between the palato-quadrato on the one hand and the lamina orbitonasalis (antorbital process) and the subocular cartilage on the other. The latter element has not yet appeared at this stage, but it will be described below. At later stages I have also observed traces of chondrification in this mesenchymatous connexion, thus confirming Edgeworth's observations. If Sewertzoff and Disler's view is correct, it is important in showing that the mandibular arch was once divided into four elements, like the branchial arches. The evidence from *Scyllium*, however, does not seem to be sufficient to decide this matter.

Stage 9 (35 mm., Plymouth, figs. 13, 14, Pl. 34).

The embryo illustrated in figs. 13 and 14, Pl. 34, has been dissected so as to obtain a better view of the neurocranium by removing the visceral arches. Cartilage is now spreading between the rostrum, the trabeculae, and the base of the laminae orbitonasales, to form a fairly extensive plate. This plate is notched on each side at its anterior edge, for the exit of a vein.

The lamina orbitonasalis now shows features of considerable interest. Extending upwards from it and from the trabecula at its base is a plate of cartilage which is curved, so that it presents a convex face anteriorly towards the nasalsac and a concave face posteriorly towards the orbit. This plate is perforated at about its centre by a foramen through which a vein passes from the orbit into the future cavity of the nasal capsule. Now



TEXT-FIGS. 6-25.

Selected transverse sections through an embryo of *Scyllium canicula* about 35 mm. long (G. R. de Beer, 35):

Fig. 6.—Section 3-1-1.

Fig. 7.—Section 4-1-2.

Fig. 8.—Section 4-4-1.

Fig. 9.—Section 4-4-10.

Scyllium has lost its profundus nerve, but a comparison with *Heterodontus* shows that this foramen is the one through which the profundus nerve typically leaves the orbit for the nasal capsule. In other words, this foramen has the relation of an orbitonasal fissure. Owing to the curvature of the plate through which it is pierced, when seen in dorsal view, this orbitonasal foramen gives the appearance of being a notch in its anterior face.

The question arises as to the nature of this cartilaginous plate, and, in particular, of that part of it which is situated median to the orbitonasal foramen. In other vertebrates, the orbitonasal foramen or fissure lies between the planum antorbitale of the nasal capsule and the side wall of the skull in the form of the orbital cartilage. Now, applying these relations to *Scyllium*, it would seem that that portion of the plate which lies median to the orbitonasal foramen is to be regarded as a preoptic root of the orbital cartilage, springing from the trabecula; while the cartilage lateral to the orbitonasal foramen is the planum antorbitale, springing from the lamina orbitonasalis or ethmoid process. The orbital cartilage is therefore represented by two separate portions: a preoptic root developed in close association with the planum antorbitale, and a large plate developed in association with the pila antotica. These two portions will eventually fuse together on each side, and already at this stage an irregular piece of cartilage is present near the future point of fusion. In connexion with the previous stage mention was made of the subocular cartilage. This structure arises as a band of cartilage below the lateral edge of the trabecula and parachordal. In *Scyllium*, as in *Pristiurus* according to Sewertzoff's (1899) descriptions, the subocular cartilage appears to chondrify from two centres. The anterior centre is close to the lamina orbitonasalis and is involved in the connexion with the palato-quadrate; the posterior centre is in the neighbourhood of the foramen prooticum. Here the subocular cartilage wraps round the orbital artery and is attached to the skull-wall in front of and behind it, with the result that the artery emerges through a foramen on to what will become the upper surface of the subocular shelf. Eventually the anterior

and posterior portions of the subocular cartilage join, forming a continuous subocular shelf, which becomes attached to the lateral edge of the trabecula and parachordal between the lamina orbitonasalis and the auditory capsule. But, for a long time, the subocular cartilage is mostly free, as the transverse sections show.

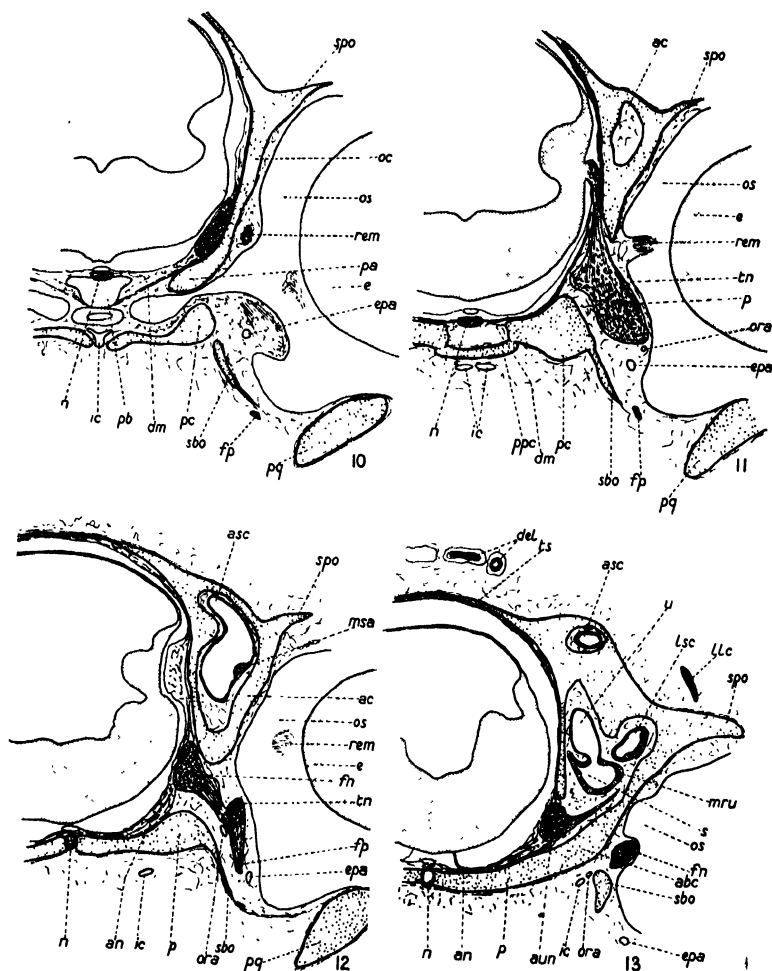
The foramen prooticum has become subdivided into the openings for the main branches of the trigeminal and facial nerves, and the separate openings for the superficial ophthalmic branches of these nerves.

The carotid arteries now enter the cranial cavity through a foramen formed by the development of a precarotid commissure joined on each side on to the postpituitary commissure. In the vertebral column, the basidorsals and basiventrals of corresponding segments are becoming joined. The occipital arches have come into contact with the posterior wall of the auditory capsule, thus enclosing the fissura metotica to form a foramen jugulare, through which the vagus nerve passes. This nerve is separated from the glossopharyngeal by the approximation of the under surface of the auditory capsule to the upper surface of the lamina hypotica. The notch for the glossopharyngeal is just behind the articular facet for the hyomandibula. The basicapsular fenestra has been obliterated.

Stage 10 (36 mm., Plymouth, figs. 15, 16, Pl. 34; fig. 17, Pl. 35).

At this stage the outlines of the nasal capsule are marked out, and there is now a thin strand of cartilage connecting the rostrum with the planum antorbitale, forming the rudiment of the front and side walls of the nasal capsule. The nasal cartilages are still free, on the under surface of the future capsule. Cartilaginous connexion has almost been established between the main body of the orbital cartilage and the preoptic root. In the embryo illustrated in figs. 15 and 16, Pl. 34, the positions of the thin film of cartilage connecting the lamina orbitonasalis with the basal process of the palato-quadrate, and of the anterior portion of the subocular cartilage, are indicated with dotted lines. A new feature at this stage is the supraorbital cartilage,

which projects laterally from the dorsal edge of the orbital



TEXT-FIGS. 10-13.

Fig. 10.—Section 5-1-3.

Fig. 12.—Section 5-2-7.

Fig. 11.—Section 5-1-8.

Fig. 13.—Section 5-3-9.

cartilage and taenia marginalis, and is continued back on to the lateral surface of the auditory capsule. It is in the latter

region that this cartilage is best developed, and it forms an arch through which the lateral line canal passes. •

The most interesting feature shown by this stage concerns the region between the polar cartilages. As in previous stages, there is a postpituitary commissure and a precarotid commissure, which, between them, enclose the carotid arteries in their foramen and separate them from the remainder of the hypophysial fenestra. In addition to these two transverse cartilaginous bars, there are now a pair of processes which project towards one another from the foremost points of the parachordals, dorsal to the polar cartilages, and in intimate relation to the dura mater, in which the notochord itself is also enclosed. These processes, which eventually meet one another, form the true dorsum sellae or front edge of the parachordal plate, beyond which the notochord projects a little way into the plica encephali ventralis. These relations are further shown in Text-figs. 4 and 5.

Up till now, the front ends of the parachordals had been divergent from one another. But now that they become connected by a transverse dorsum sellae, a true basicranial fenestra is enclosed. This fenestra has not previously been described in Selachians. Its existence has been confirmed in several preparations of *Scyllium* made by the van Wijhe method, and also in serial sections. It eventually becomes completely obliterated by the extension of the parachordals. The anterior edge of the dorsum sellae also seems to grow forwards, so that in later forms it definitely overhangs the pituitary fossa or sella turcica from behind. A consequence of the formation of the postpituitary commissure and of the dorsum sellae is that the pituitary vein becomes enclosed in its cartilaginous interorbital canal.

Fig. 17, Pl. 35, is a drawing of a ventral view of the nasal cartilages and of the mandibular and hyoid arches. It may be remembered that Parker (1878) referred to the nasal cartilages as labial cartilages: a view which it is difficult to support. Between the ventral ends of the ceratohyals the basihyal has appeared, and it is pierced by a foramen through which the stalk of the thyroid gland passes. The existence of this

foramen strongly suggests that the basihyal is really of paired nature.

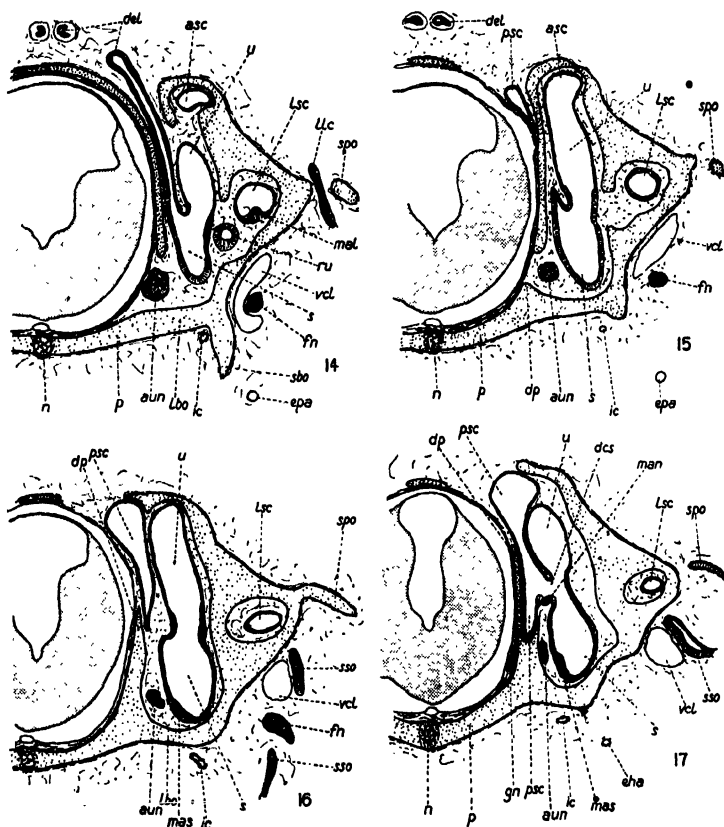
Stage 11 (36 mm., Naples, fig. 18, Pl. 85).

This embryo has been drawn from the left side and slightly from behind, to show the various canals in the auditory capsule as seen by transparency, and to show the relations of the occipital arch to the auditory capsule, and the various elements of the vertebral column. In particular, it is possible to see how the posterior canal of the auditory capsule (it can scarcely be called a semicircular canal, for it occupies a complete circle) has bulged backwards over the lamina hypotica, so that the points of exit of the glossopharyngeal and vagus nerves are fairly widely separated.

Stage 12 (37 mm., Plymouth, figs. 19, 20, Pl. 86).

In this embryo, continuity has been established between the main body of the orbital cartilage and its preoptic root. There is still, however, a vacuity between the preoptic root and the main portion of the planum antorbitale, dorsal to the orbitonasal foramen, due to delayed chondrification. The suborbital and supraorbital cartilages are better developed than in previous stages, and the film of cartilage between the lamina orbitonasalis and the palato-quadrates is still present. The supraorbital cartilage encloses between itself and the dorsal edge of the orbital cartilage the twigs of the superficial ophthalmic branch of the facial nerve, innervating the supraorbital lateral line canal. The existence of these foramina, and of the arch further back through which the lateral line canal itself passes, is evidence in favour of the view that the supraorbital cartilage, like the suborbital cartilage, has an independent origin, and becomes attached to the orbital cartilage. The postpituitary commissure shows a small variation in that it is still unattached to the polar cartilages. The precarotid commissure, and the processes from the foremost ends of the parachordals which go to form the dorsum sellae, are present. A labial cartilage has appeared in the upper jaw, by the side of and

close to the anterior portion of the palato-quadrate. Its partner in the lower jaw does not appear until later.



TEXT-FIGS. 14-17.

Fig. 14.—Section 6-1-3.

Fig. 16.—Section 6-2-1.

Fig. 15.—Section 6-1-8.

Fig. 17.—Section 6-2-6.

Stage 18 (45 mm., Naples, figs. 21, 22, 23, 24, Pl. 37).

This stage is the oldest studied in this paper, and it leads on easily to the conditions in the adult. But, like all the Naples material, it is in some respects more retarded than smaller embryos from Plymouth; for instance, the basidorsals and

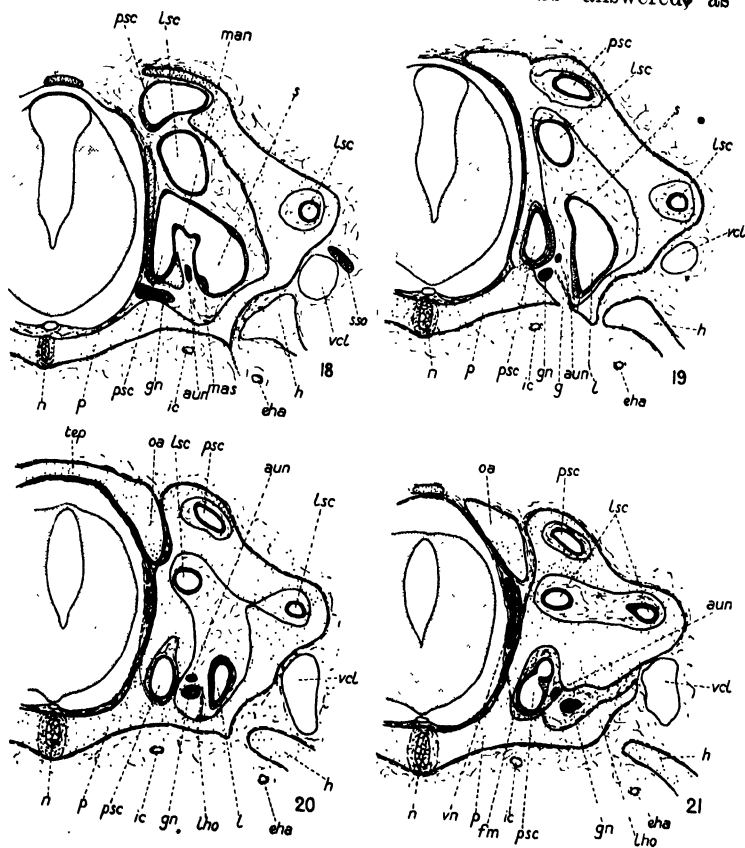
basiventrals are still separate, whereas they were fused in their respective segments in the embryo described in stage 9.

The nasal capsule is further developed, and a considerable portion of its front and side walls is now formed. A pair of cartilages rise up from the rostrum on each side, forming the median wall of each nasal capsule, and, running upwards and forwards, are attached to the dorsal part of the front wall of the capsule. From near their point of attachment to this wall, the lateral rostral processes project forwards. The original rostral cartilage extends forwards as the median rostral process, and, dorsal to it, a shorter and more blunt process extends forwards between the median walls of the nasal capsules (the supra-rostral process). The nasal cartilage is unattached to the front wall of the nasal capsule, and its outermost prong is now detached from it, and forms a small separate cartilaginous curved strut round the outer and hinder sides of the external nostril.

The postpituitary and precarotid commissures and the processes forming the dorsum sellae are as in previous stages, but the remainder of the hypophysial fenestra is beginning to be obliterated by islets of cartilage which will contribute to form the trabecular plate. The basicranial fenestra is also reduced by further extension of the parachordal cartilage. A roof is now present in the form of a small quadrangular cartilage. The two hind corners of this cartilage are continuous with the occipital arches, thus forming a tectum posterius. The two front corners are almost continuous with processes which extend towards them from the roofs of the auditory capsules. When these establish connexion, a tectum synoticum will have been formed. On each side of the quadrangular cartilage, therefore, there is an unchondrified region which gives rise to the parietal fossa of the adult skull, and which lies immediately dorsal to the vacuity in the median wall of the auditory capsule through which the posterior (semi-)circular canal bulges.

The spiracular cartilage is now present as a thin transverse plate immediately in front of the spiracle. As Ridewood (1895) showed, this cartilage has nothing to do with the ligament suspending the palato-quadrate. This ligament was erroneously termed the prespiracular ligament (whereas it is in reality

postspiracular) by Parker (1878). Huxley (1876) showed that the spiracular cartilage of *Heterodontus* answered, as



TEXT-FIGS. 18-21.

Fig. 18.—Section 6-3-1.

Fig. 20.—Section 6-4-1.

Fig. 19.—Section 6-3-5.

Fig. 21.—Section 6-4-8.

regards its relations, to the otic process of the frog, with which he regarded it as homologous.

The visceral arch skeleton is now complete; consisting of palato-quadrate, Meckel's cartilage, hyomandibula, ceratohyal, five pharyngobranchials (the last two being joined), five epibranchials, five ceratobranchials, three hypobranchials

corresponding to the first three branchial arches, and a median cartilage representing the fused hypobranchials of the last two arches, and a median basihyal which is still perforated by a foramen for the remains of the thyroid stalk. Four dorsal and three ventral extrabranchial cartilages are present, as well as the hyal and branchial rays. The hyal rays are grouped into two lots, the more dorsal being attached to the hyomandibula and the more ventral to the ceratohyal.

It is interesting to compare the median views of longitudinal sections of the skull at this stage with those of the adult (figs. 24 and 25, Pl. 37). The embryo illustrated in fig. 24, Pl. 37, is slightly younger than that in figs. 21 to 23, Pl. 37. It has been dissected and cut longitudinally so as to show the median surface of the auditory capsule. The median wall of the capsule is not complete, but an important part in its formation is taken by the dorsal process of the parachordal, which separates the auditory from the glossopharyngeal nerves. It is also possible to see how the posterior wall of the capsule has become attached to the occipital arch, and overlies the lamina hypotica, thus obliterating the fissura metotica between the glossopharyngeal and vagus nerves. In the adult, the median wall of the auditory capsule has been completed, except of course for the auditory foramen, the endolymphatic foramen, and for a vacuity just behind the latter through which the posterior canal of the membranous labyrinth bulges. This is the region of the parietal fossa, where the roof of the brain-case dips down between the tectum posterius and the tectum synoticum, and there is a vacuity in the roof on each side. As may be seen from the transverse sections, the posterior canal vacuity in the wall of the auditory capsule communicates, not with the cranial cavity, but with the tissues outside it, for the vacuity is shut off from the cranial cavity by the dura mater, which in this region is unchondrified. The glossopharyngeal foramen is not morphologically a perforation of the median wall of the capsule, but a remnant of the fissura metotica, between the capsule and the parachordal plate. The relations of the cartilages to the canal for the glossopharyngeal nerve are also shown in figs. 26-8, Pl. 36, in which it may be seen how the lamina hypotica forms

as it were a false bottom to the hinder part of the auditory capsule.

Figs. 24 and 25, Pl. 37, also serve to show how the post-pituitary and precarotid commissures and the processes forming the dorsum sellae combine to form the well-marked ridge of the adult, overhanging the pituitary fossa (sella turcica) from behind, and enclosing the pituitary vein in the cartilaginous interorbital canal.

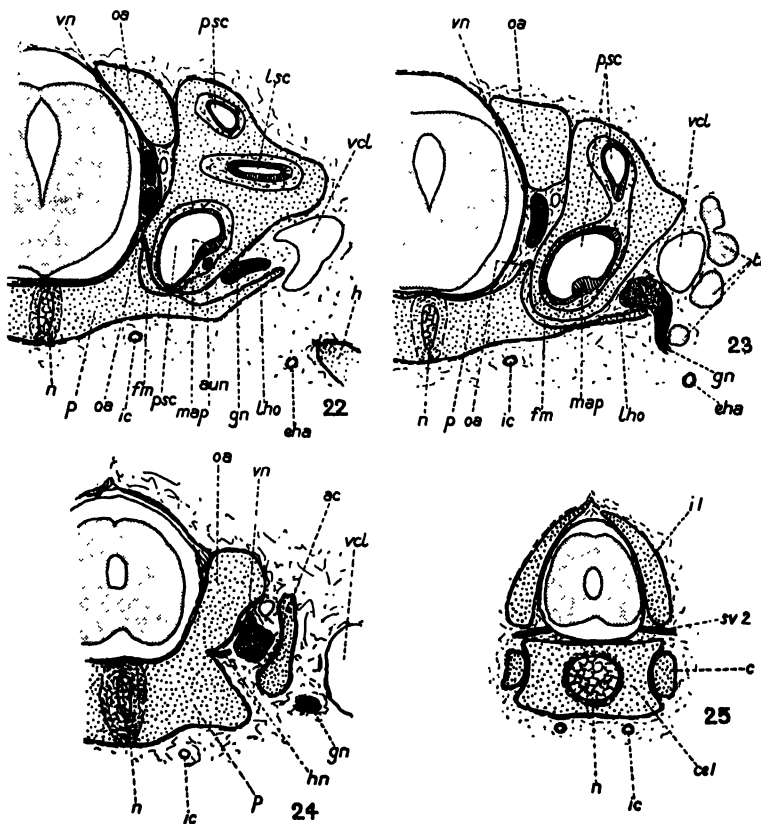
The Auditory Capsule.

The study of the auditory capsule of *Scyllium* throws light on two problems of interest, concerning the median wall of the capsule and the relations to the capsule of the glossopharyngeal nerve.

Gegenbaur (1872, p. 49) described the pit in the roof of the skull at the point towards which the anterior and posterior semicircular canals converge as the parietal fossa. At the bottom of this pit he said that there were two openings on each side leading into the auditory capsule, but gave no further details concerning them. Parker (1878, p. 208) refers simply to the 'aqueducts of the vestibule', yet his fig. 4 on Pl. xxxviii appears to show the correct relations, although unlabelled. Wells (1917) and Daniel (1922) refer to openings for perilymphatic spaces, by which they mean the vacuities through which the posterior semicircular canals protrude from the capsules. Quite recently, Norris (1929) has alluded to these vacuities, and traced the history of their study at the hands of Geoffroy (1780), Monro (1785), Scarpa (1789), and Weber (1820). Scarpa regarded the vacuities as equivalents of the fenestra ovalis of the ear of higher forms; Weber considered them as the fenestra rotunda.

At the outset, it is necessary to realize that the auditory organ of Selachians, as admirably set forth by Retzius (1881), differs in certain remarkable respects from that of other vertebrates. In particular, the posterior semicircular canal is worthy of attention. Instead of its anterior limb being joined on to the posterior limb of the anterior semicircular canal to form a crus commune, the posterior canal in Selachians practically forms

a complete circle or ring, apposed to the postero-medial surface of the sacculus and utricle, and with the cavity of which it communicates by means of the ductus canalis semicircularis



TEXT-FIGS. 22-25.

Fig. 22.—Section 6-4-8.

Fig. 24.—Section 7-2-5.

Fig. 23.—Section 7-1-3.

Fig. 25.—Section 7-4-4.

posterioris. These relations are shown in the text-figures of sections which accompany this paper. The lateral portion of the posterior canal is lodged in the cartilaginous capsule, but its median portion protrudes through the vacuity in the median

wall of the capsule. This vacuity (the posterior canal vacuity), places the cavity of the auditory capsule in communication with the surrounding regions, but not with the cranial cavity. As may easily be seen in the sections, the part of the posterior canal which bulges out through the vacuity is excluded from the cranial cavity by the dura mater, which in this region is not reinforced by cartilage. When in the dry skull a seeker is passed through the parietal fossa into the cranial cavity, it does not respect the boundaries of the true cranial wall. Morphologically the parietal fossa opens only into the auditory capsule. In *Scyllium* the vacuity through which the posterior semi-circular canal protrudes appears to be occluded only by the membranous wall of the canal itself, and not by a definite membrane. Readers are referred to Norris's paper (1929) for an account of the conditions in other forms.

The existence of the posterior canal vacuity brings it about that the median wall of the auditory capsule is incomplete for a certain short distance, and in this region there is therefore no contribution from the auditory capsule to the formation of the roof of the skull. Posteriorly to the vacuity the roof of the skull is formed by the tectum posterius; anterior to it is the tectum synoticum. The parietal fossa owes its existence to the presence of the posterior canal vacuity.

Wholly distinct from the posterior canal vacuity, yet like it opening into the parietal fossa, is the foramen for the ductus endolymphaticus. It lies in front of the posterior canal vacuity, the ductus endolymphaticus and the posterior canal being separated by a wall of cartilage. In *Heterodontus* Norris (1929) states that the posterior canal vacuity has probably become confluent with the endolymphatic foramen, a conclusion with which I agree.

Gegenbaur (1872) realized that the glossopharyngeal nerve on its passage through the wall of the skull traversed the cavity of the vestibule of the auditory capsule. Since primitively the nerve must have passed behind the capsule, it is of interest to see how the conditions which prevail in Selachians may be explained. The problem is identical with that concerned with the passage of the facial nerve through the anterior region of

the auditory capsule in *Urodela* (Goodrich, 1930), and is to be explained in the same way. Briefly what has happened is that a lateral shelf from the parachordal cartilages has extended beneath the floor of the auditory capsule. Originally the glossopharyngeal nerve passed out between this shelf (*lamina hypotica*) beneath, and the floor of the capsule above, in a space which represents the *fissura metotica*, and behind the basi-vestibular commissure, represented in *Selachians* by the dorsal process of the parachordal plate. Then the floor of the auditory capsule became reduced and fenestrated while the *lamina hypotica* persisted, and formed a vicarious lower cartilaginous boundary to the capsule. In one place the *lamina hypotica* does not quite reach the true floor of the capsule, just in front of it, with the result that there is a gap in the cartilage, as may be seen in Text-fig. 19. Behind this point the lateral edge of the *lamina hypotica* is confluent for a short distance with the lower edge of the outer wall of the capsule, but histological differences in the cartilage mark the limit to which each extends. Farther back again, the *lamina hypotica* extends freely to the side beneath the capsule which in this region has its true floor chondrified again. The *lamina hypotica* here forms the lower border of the glossopharyngeal notch. Meanwhile the cavity of the capsule and that portion of the *fissura metotica* which acts as a glossopharyngeal canal are thrown into one, and the boundary between them is indicated only by some remnants of membrane. At the same time the capsule has bulged backwards to accommodate the large posterior canal, and so it happens that the glossopharyngeal nerve in *Selachians* seems to penetrate the cavity of the auditory capsule itself, passing in front of the posterior canal, on the inner hinder side of the *lagena*, and to run for a short distance close to the posterior branch of the auditory nerve. A minute examination of sections reveals traces of the obliterated *fissura metotica*, through which both glossopharyngeal and vagus nerves typically pass.

The disentangling of the relations of the glossopharyngeal nerve to the auditory capsule in *Selachians* is important in connexion with the conditions in the *Teleostomes*. As is well known, in the latter animals the median wall of the auditory

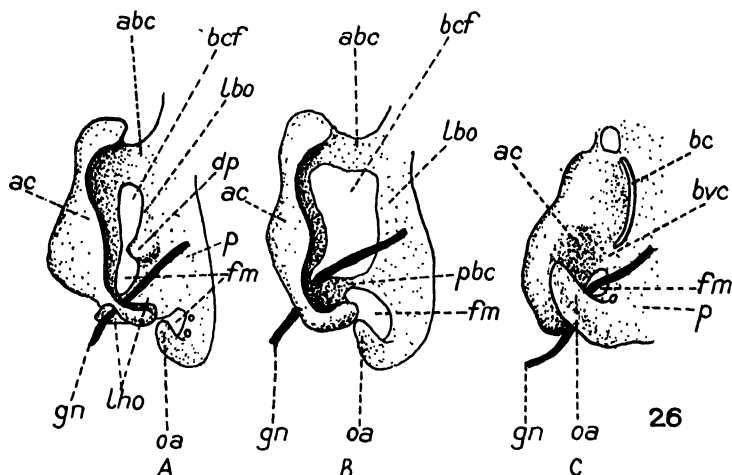
capsule is much reduced. Now, in the young embryo of *Acipenser stellatus*, the fissura metotica is present, the vagus and glossopharyngeal nerves traversing it in typical manner. At a later stage, however, the fissura metotica is divided by a fusion of the lateral edge of the parachordal with the median wall of the auditory capsule into an anterior glossopharyngeal foramen and a posterior typical vagus foramen. A true basi-vestibular commissure is present, forming the junction between parachordal and capsule, anterior to the glossopharyngeal. The fusion alluded to in the previous sentence is really formed by the cartilage which gives rise to what Gaupp (1906) has called the posterior basicapsular commissure. This commissure in *Salmo* lies behind the glossopharyngeal nerve, and for some time there is no true basivestibular commissure, with the result that the glossopharyngeal nerve is enclosed in the basicapsular fenestra. The basicapsular fenestra of *Salmo* therefore contains a portion of the fissura metotica, and is not strictly equivalent to the basicapsular (or basicochlear) fissure of higher vertebrates, which is situated wholly in front of the basivestibular commissure, contains therefore no part of the fissura metotica, and is not traversed by the glossopharyngeal nerve (Text-fig. 26).

The absence of a median cartilaginous wall to the auditory capsule, and the inclusion of the glossopharyngeal nerve in the basicapsular fenestra in *Salmo*, are responsible for the passage of the nerve through the capsule in that animal, for the membranes indicative of the true boundaries of the capsule have mostly broken down. This condition would appear, however, not to be universal in the Teleostei, for in *Gasterosteus*, Swinerton (1902) states that the cartilage of the auditory capsule 'is continuous both with the lateral process of the occipital arch and with the postero-lateral border of the mesotic region, thus forming a complete boundary around the exit for the ninth and tenth nerves'. Here, then, the glossopharyngeal nerve clearly does not traverse a basicapsular fenestra, but accompanies the vagus through the fissura metotica. *Gasterosteus*, therefore, lacks a posterior basicapsular commissure, and the continuity of the cartilage of the auditory capsule with the 'postero-lateral

border of the mesotic region' must represent a true basivestibular commissure.

THE RELATIONS OF THE JAWS TO THE BRAIN-CASE

As is well known, Huxley (1876) in his classical memoir effected a subdivision of the types of suspension of the jaws into



TEXT-FIG. 26.

Diagram to illustrate the relations between the glossopharyngeal nerve and the auditory capsule in: *A*, the Selachian (*Scyllium*); *B*, the Teleost (*Salmo*); and *C*, the Mammal (*Lepus*).

three categories: viz. amphistylic, hyostylic, and autostylic. As it is matter of great importance to ascertain exactly what he meant, some extracts from his paper will be quoted here.

(1) 'In the amphistylic skull the palato-quadrate cartilage is quite distinct from the rest of the skull; but it is wholly, or almost wholly, suspended by its own ligaments, the hyomandibular being small and contributing little to its support' (e.g. *Notidanus* = *Heptanchus*).

(2) 'The palato-quadrate cartilage is no longer continuous with the chondrocranium (though the bony elements of that arch (i.e. mandibular) may unite suturally with those of the skull, as in the *Plectognathi*), but is, at most, united with it by ligament.

Moreover, the dorsal element of the hyoidean arch, or the hyomandibular, usually attains a large size and becomes the chief apparatus of suspension of the hinder end of the palato-quadrate cartilage with the skull. Skulls formed upon this type, which is exemplified in perfection in Ganoidei, Teleostei, and ordinary Plagiostomes, may therefore be termed *hyostylic*.'

(3) 'The part of the palato-quadrate cartilage which is united with the skull, between the exits of the fifth and second nerves, answers to the "pedicle of the suspensorium" of the amphibian, while its backward and upward continuation on to the periotic cartilage corresponds with the otic process. As in the *Amphibia* and in the higher *Vertebrata*, the mandibular arch is thus attached directly to the skull by that part of its own substance which constitutes the suspensorium. It may thus be said to be *autostylic*.'

While there can be little ambiguity about the term *hyostylic* (although it needs to be analysed further), the same is not true of the terms *autostylic* and *amphistylic*. As regards the *autostylic* condition, it may mean simply that the mandibular arch is not dependent on the hyoid arch for part or most of its suspension from the brain-case, or it may mean that the mandibular arch is fused by means of one or more of its own processes on to the cartilage of the brain-case. It is true that living fish do not present one of these alternatives without the other, but as the former may be a primitive feature and the latter is probably secondary, there is reason to suspect that in extinct forms these alternatives were not inseparably associated. It is not quite clear from Gadow's (1888) descriptions which meaning he attached to the term 'simple *autostylic* form'.

It would seem that Huxley himself laid most stress on the fusion of the jaws with the brain-case, for, having finished with the description of the *autostylic* forms, he goes on to say that in all other fishes 'the palato-quadrate cartilage is no longer continuous with the chondrocranium', suggesting therefore that continuity is an essential feature of his *autostylic* category. This view of the meaning of the term *autostylic* is also shared by Smith Woodward (1898) and Gregory (1904):

For the sake of clearness, the term *autostylic* will be taken

to mean this alternative, viz. that condition in which the mandibular arch is fused to the brain-case in the adult form. In such forms, the hyomandibula plays no part in the suspension, but this would seem to be because the hyoid arch has been reduced from a previous large and suspensorial condition, and not because the hyoid arch has not yet acquired a suspensorial function.

A difficulty arises from the fact that the palato-quadrate cartilage has no less than four processes, viz. ethmoid, basal, ascending, and otic, by means of which suspension may be effected, and it becomes necessary to distinguish between them. *Heptanchus* is amphistylic and attached by the basal and otic processes; *Heterodontus* (Cestracion) is attached only by the basal process (and of course the hyomandibula); *Salmo* is attached only by the ethmoid process (and hyomandibula).

Another question which Huxley's classification raises is due to the fact that it is essentially a functional classification, since it is primarily based, not on whether a particular structure, say the otic process, exists, but on whether such a structure actually serves in the suspension of the jaws. Now in some forms the otic process exists, but it is too small to effect a functional suspension, as in *Scymnus* and *Amia*, or it may be detached from the palato-quadrate cartilage to form the spiracular cartilage, as in *Scyllium*. Clearly *Scymnus*, *Amia*, and *Scyllium* cannot be placed in the same group as *Heptanchus* on Huxley's classification, and yet the possession of an otic process (albeit diminutive) deserves a recognition of the affinity expressed between these forms and *Heptanchus* by the possession of a homologous structure. It seems, therefore, that while Huxley's scheme may most certainly be retained in order to indicate the type of suspension of the jaws, another classification based purely on morphological considerations is necessary to express the phylogeny of the suspension.

An attempt was made to revise the classification of the types of jaw-suspension by Gregory (1904), according to the following system.

<i>Type.</i>	<i>Palato-quadrate.</i>	<i>Hyomandibula.</i>	<i>Term.</i>
Ancestral	Little or no attachment	Not suspensorial	Palaeostylic
Chlamydoselache	Loose articulation (basal)	Suspensorial	Hyostylic
Scyllium	No articulation	Suspensorial	Hyostylic
Heterodontus	Close articulation (basal)	Suspensorial	Hyostylic*
Heptanchus	Close articulation (basal + otic)	Suspensorial	Amphystylic
Raja	Quite free	Suspensorial	Euhyostylic
Acipenser	Quite free	Suspensorial	Methyostylic
Polypterus	Articulation (ethmoid)	Suspensorial	Methyostylic
Salmo	Articulation (ethmoid)	Suspensorial	Methyostylic
Chimaera	Close fusion	Not suspensorial	Holostylic
Ceratodus	Fusion (basal, ascending, otic)	Not suspensorial	Autostylic
Amphibia	Fusion (ethmoid, basal, ascending, otic)	Not suspensorial	Autostylic

A more recent classification is that of Edgeworth (1925) which runs as follows:

Scyllium	Hyostylic.
Squalus	Amphistylic and streptostylic.
Heptanchus	Autostylic and streptostylic.
Heterodontus	Amphistylic.
Acipenser	Hyostylic.
Polyodon	Hyostylic.
Polypterus.	Hyostylic.
Amia	Amphistylic.
Lepidosteus	Amphistylic.
Salmo	Autostylic anteriorly and hyostylic posteriorly.

Stannius (1856) coined the terms 'streptostylic' and 'monimostylic' to express the mobility of the quadrate in the bony skull. Fürbringer (1900 and 1904) applied these terms, as does Edgeworth, to the cartilaginous skull, to express the freedom or attachment of the palato-quadrate to the brain-case.

Fuchs (1915) has pointed out that this extension of the use of the terms carries with it an alteration in their meaning. And since Versluys (1912) has shown that in the bony skull streptostyly and monimostyly only constitute special cases of a more comprehensive phenomenon, i.e. 'kinetism' and 'akinetism', it seems inadvisable to use the same expression streptostylic to denote the freedom of the palato-quadrate in the (cartilaginous) skull of the Selachian and the mobility of the quadrate in the (bony) skull of the Reptilian. The latter meaning has priority.

But be this as it may, Fürbringer regarded the mobility of the palato-quadrate as the primitive condition ('It is so-to-speak a morphological necessity', 1904, p. 585), whereas Edgeworth thinks that the fixed palato-quadrate is the more primitive. Before the question of the suspension of the jaws in the cartilaginous skull can be tackled, attention must be turned to some points of general principle. These are concerned with (1) the value of embryological evidence on questions of phylogeny, and (2) problems of phylogeny and nomenclature.

Edgeworth is impressed by the fact that the joint between the palato-quadrate cartilage and the neurocranium in *Squalus* and *Lepidosteus* in the region of the basal process is preceded in development by cartilaginous continuity; the same being probably true of *Heptanchus* also. This leads him to conclude that 'Selachii, Batoidei, and Teleostomi are descended from autostylic and monimostylic ancestors in which there was a pterygo-quadrate united to the Chondrocranium at three points' (1925, p. 257). This conclusion presupposes that conditions which are embryonic in development must represent ancestral adult conditions in phylogeny. Elsewhere (de Beer, 1930 A) I have endeavoured to draw attention to the fact that this conclusion is not sound, whether by reference to empirical evidence, or by logical deduction from other available evidence concerning embryology and evolution. It is no more legitimate to say that the adult ancestral vertebrates were autostylic because in early stages of development of some forms a transient cartilaginous continuity exists between the palato-quadrate and the neurocranium, than it would be to assert that the ancestral

adult nervous system was a solid rod because it develops as a solid rod in *Petromyzon*, *Lepidosteus*, *Teleostei*, and *Lepidosiren*.¹

There is then no proof that the ancestral vertebrates had jaws fused with their brain-case, and the facts that the only such forms are those which are most specialized in other respects, and that the postmandibular visceral arches are not confluent with the axial skeleton, point rather to the view that the splanchnocranium and neurocranium were originally distinct.

Now turning to questions of phylogeny and nomenclature, it

¹ The same criticism may be made in respect of the conclusions which Edgeworth has based on his admirable researches into the method of origin of the masticatory muscles. That the primordium of the muscles of the mandibular arch in non-Dipnoan fish should be divided into a dorsal (*Constrictor i dorsalis*) and a ventral portion (*Adductor mandibulae*), while in Dipnoi and Amphibia the primordium is not so divided (a point which, incidentally, is contested by Luther, 1914), i.e. remains in the condition through which the non-Dipnoan fish pass, is no evidence that the Dipnoi and Amphibia are phylogenetically more primitive than the non-Dipnoan fish. It cannot be argued that because Caducibranchiate *Urodela* pass through a stage at which *Perennibranchiates* remain, the latter are the more primitive. Indeed, the secondary nature of the retention by adult descendants of characters which were larval or embryonic in the ancestors, is becoming more and more clear. This paper is not concerned with muscles, but this matter must be mentioned, for Edgeworth seeks to substantiate his view that the ancestral vertebrates were autostylic by means of the conclusions which he has drawn from his investigations into the development of muscles. As they stand, these conclusions are inadmissible, and they receive final refutation from his further attempt to make the evidence from the development of the extrinsic eye-muscles coincide with them. In *Selachians* the eye-muscles develop, as is well known, from the first three somites, and they preserve their innervation by the ventral nerve-roots of these segments. In *Teleostomes* the development is more obscure, and Edgeworth believes that they develop from the first and second somites only, while in Dipnoi and Amphibia he derives them from the first somite alone. These results lead him to conclude (1926, p. 32) that 'the conditions in *Teleostomi* and *Plagiostomi* may be considered secondary and tertiary modifications of those in Dipnoi, *Urodela*, and *Anura*'. As this view is inconsistent with van Wijhe's theory, Edgeworth (1928, p. 46) rejects the latter. It is only because Edgeworth has not dissociated the conceptions of primitive and secondary in phylogeny from those of early and late in ontogeny that he has thus been led to read the series *Selachii-Teleostomi-Amphibia* in a direction which is the opposite of that to which all the evidence of zoology and palaeontology point.

is clear that the latter is most instructive when based on the former. Further, the establishment of a phylogenetic series cannot be based satisfactorily on a consideration of only one or two sets of structures, although a single set of structures may be very useful in showing when a series is not phylogenetic. All the evidence of zoology shows that the Chondrichthyes are at a lower and more primitive level of evolutionary organization than the Osteichthyes, which, in turn, are at a lower level than the Tetrapoda. In order to obtain the evidence which the suspension of the jaws presents on this problem, it is necessary, therefore, to examine the different structures which may be concerned with suspension in the various forms, to establish their homology or non-homology by the morphological criterion of their geometrical relations to neighbouring structures, and to compare the results thus obtained. For reasons stated above, embryological evidence cannot be relied upon, since it is not possible to assert that ontogenetic features have phylogenetic significance.

It may now be taken as accepted that Huxley's view is correct in regarding the basal process of the palato-quadrates as the original dorsal end of the mandibular arch. Swinnerton (1902) and Sushkin (1927) have stressed the fact that the basal process is very persistent among the various types of fish, and Goodrich (1930) has summarized the evidence from other quarters in favour of Huxley's view. For a long time it seemed doubtful whether the orbital process of the Selachian represented an ethmoid or a basal process. But the existence of an ethmoid articulation in front of the orbital process in *Scymnus* and *Chlamydoselache*, together with the fact that the ethmoid process forms the most anterior extremity of the palato-quadrates cartilage in Teleostomes and higher forms, renders it reasonably certain that the orbital process of Selachians is a basal process. This process may be situated far forward as in *Scyllium*, or far back as in *Scymnus*, depending on the relative lengths of the jaws and the brain-case.

Regan (1923) and Norman (1926) believe that the basal articulation of *Lepidosteus* is not primitive, but has been secondarily acquired. But in view of the prevalence of this

articulation throughout the vertebrates, and of the fact that its morphological relations in *Lepidosteus* are identical with those of other forms, its independent acquisition by *Lepidosteus* is hard to believe.

Before considering the distribution of the basal and other processes in the various groups, attention must be turned to the question as to whether the anterior visceral arches, i.e. the mandibular and hyoid arches, ever showed the division into four elements characteristic of the more posterior (or branchial) arches. The palato-quadrate and hyomandibula seem to represent the epal elements of their respective arches, just as Meckel's cartilage and the ceratohyal appear to represent the ceratal elements. If such be the case, then it may be supposed that the pharyngomandibular, pharyngohyal, hypomandibular, and hypohyal elements originally existed. On the other hand, if the anterior visceral arches never were divided into four pieces but only into two (i.e. palato-quadrate and Meckel's cartilage—hyomandibula and ceratohyal), as Gegenbaur (1872) believed, the above-mentioned elements could not have existed.

Now, paired cartilages which seem to be hypohyals are present in *Salmo* and in *Lacerta*, and the so-called 'hyoid' of the rabbit shows evidence of paired origin. Among *Selachii*, hypohyals have been described in *Laemargus* by White (1895), and in *Heptanchus* by Braus (1906) and Fürbringer (1908). A pharyngohyal is present in *Callorhynchus* (Schauinsland, 1908), and Luther (1909) claims to have found it in *Stegostoma*, *Mustelus*, and *Galeus*. There seems, therefore, to be considerable evidence in favour of the view that the hyoid arch was originally similar to the more posterior arches in being divided into four elements. It is probable that the disappearance of the pharyngohyal was associated with the acquisition by the hyoid arch of the function of suspending the jaws, and it is interesting to note that in *Callorhynchus*, the only form in which the pharyngohyal is at all well developed, the hyoid arch has no suspensory function.

As regards the mandibular arch, a hypomandibular has been described in *Heptanchus* and *Laemargus* by White (1895), and in *Hexanchus* by Fürbringer (1908) and Sewert-

zoff (1927). Jaekel (1927) has described a hypomandibular in *Acanthodes*, and van Wijhe (1922), who observed that Meckel's cartilage has two centres of chondrification in *Squalus* (and also in birds), draws attention to the possibility of interpreting his discovery in this way.

A pharyngomandibular is claimed in *Callorhynchus* by Dean (1906), and an element corresponding to it in *Scaphirhynchus* by Sewertzoff (1923) and in *Laemargus* by Sewertzoff and Disler (1924). In 1923 Sewertzoff suggested that the pharyngomandibular might be represented by the orbital (= basal) process of the palato-quadrato. In the following year, however, he altered his opinion, since he claimed that a pharyngomandibular arises separate from the orbital process in *Mustelus*, *Scyllium*, and *Squalus*, and later becomes fused on to the median side of the process. Bugajew (1929) has reported cartilages which he regards as pharyngomandibulars in three species of *Acipenser*. But the curious thing about these elements is that they do not appear to be constant. *Acipenser guldenstädti* has two such nodules on each side situated in the ligament which stretches between the basitrabecular process and the palato-quadrato; *Acipenser stellatus*, on the other hand, may have 4, 5, or 6, or 1 small and 1 large nodule.

It is clear that the case for the pharyngomandibular cannot be regarded as satisfactory, and the matter is made more difficult by Allis's (1923 A) claim, based on morphological considerations, that the pharyngomandibular is to be found in the polar cartilage of van Wijhe. In the present state of knowledge it is not possible to arrive at any definite conclusion regarding the original condition of the mandibular arch. The basal process, regarded as the original dorsal end of the arch, may therefore represent the end of the pharyngomandibular or of the epimandibular. Fortunately, this question is not of great importance for the following discussion.

The basal process is present in *Scyllium*, *Heptanchus*, *Scymnus*, *Squalus*, *Heterodontus*, and other *Squaloids*, but it has been lost in the *Batoids*; it was present in *Acanthodii* and *Pleuracanthodii* (Goodrich, 1909); there was

a basal articulation in *Osteolepidoti* (Watson, 1926), in *Coelacanthini* (Stensiø, 1922), and in *Palaeoniscoidei* (Stensiø, 1921; and Watson, 1928); remnants of a former basal articulation are found in the form of a basitrabecular process in *Acipenser* (de Beer, 1925; and Sewertzoff, 1928); a basal articulation is present in *Lepidosteus* (Parker, 1882; Veit, 1907 and 1911; and de Beer, 1926); remnants of a basal process are present in *Amia* (Pehrson, 1922; de Beer, 1926) and in *Salmo* (de Beer, 1927); the basal process fuses with the neurocranium in *Dipnoi* and some *Amphibia*.

The otic process is present in *Heptanchus*, fossil *Heterodonti* (Smith Woodward, 1898), *Acanthodii* and *Pleuracanthodii* (Goodrich, 1909), *Holocephali*; a non-articulating otic process was present in *Osteolepidoti* (Watson, 1926), *Palaeoniscoidei* (Stensiø, 1921); vestiges of an otic process are visible in *Polypterus* (Sewertzoff, 1926 A), *Lepidosteus* (Parker, 1882), *Amia* (de Beer, 1926), and *Salmo* (de Beer, 1927); the otic process is fused with the auditory capsule in *Dipnoi* and some *Amphibia*.

The ethmoid process articulates with the neurocranium in *Scymnus* (Bugajew, 1930) and in *Chlamydoselache* (Allis, 1928), and in all *Teleostomes* except *Acipenseroidei*; the ethmoid process is fused with the neurocranium in some *Amphibia*.

The ascending process is foreshadowed in *Osteolepidoti* (Watson, 1926), and it is fused with the neurocranium in *Dipnoi* and *Amphibia*.

The hyomandibula is suspensorial in all known fish except *Holocephali* and *Dipnoi*, and in no other forms.

It would seem that the above-mentioned facts may best be set into order as follows.

The most primitive suspending element is the basal process, or original dorsal end of the mandibular arch.

It is possible that in the earliest forms there was also a pre-mandibular arch, and the dorsal ends of these arches may be regarded as being loosely attached to the axial skeleton, in the form of the notochord-sheath. For such an arrangement, Gregory's term *Palaeostylic* may be used, and the *Cyclostome* condition as exemplified by *Petromyzon* may be regarded

as having been derived from it by fusion of the dorsal ends of the arches with the cartilage of the neurocranium, thus providing a firm framework for the very specialized method of feeding by means of a rasping tongue which these forms possess. Should a term be required to denote this condition, *Parautostylic* may perhaps be proposed, suggesting that the fusion of the arches with the brain-case is quite independent of the true autostyly of Dipnoi and Amphibia.

The next problem is to decide which of the two methods of suspension, by means of the otic process or of the hyomandibula, was evolved first. Fossils give little assistance here, for the earliest forms (*Pleuracanthus*, *Acanthodes*, *Cladoselache*, and *Heterodonti*) were amphistylic, and possessed both otic and hyomandibular suspensions (in addition to the basal process). An indication may, however, be obtained from a consideration of the *Holocephali*. These fish possess an otic process, but the hyomandibula is not only not suspensorial, the hyoid arch seems to have retained its primitive dorsal element, the pharyngohyal. This so-called pharyngohyal might in reality be the first pharyngobranchial, thus shifting the pharyngobranchials back one place. But a careful examination of Hubrecht's (1877) and Schauinsland's (1903) descriptions renders this unlikely. The existence of a pharyngohyal means that the *Holocephali* were descended, either from forms with an otic process and a non-suspensorial hyomandibula, or from forms in which the hyomandibula was suspensorial and subsequently lost that function and reacquired the pharyngohyal (for the presence of a pharyngohyal is inconsistent with a suspensorial hyomandibula). As the latter of these alternatives is unlikely, the only tenable opinion is that the forms from which the *Holocephali* descended had otic (and basal) processes, and a non-suspensorial hyoid arch, still retaining the pharyngohyal. The spiracular slit presumably was large, extending below the joint between hyomandibula and ceratohyal. Such a form is not separately provided for in Huxley's scheme; it was not autostylic in the adopted sense because the arches cannot have been fused with the brain-case, and it was not amphistylic since there was no support from the hyomandibula. The simplest way in which

to denote such a type would be to call it *Basiotostylic*, thus drawing attention to the elements by means of which the suspension is effected.

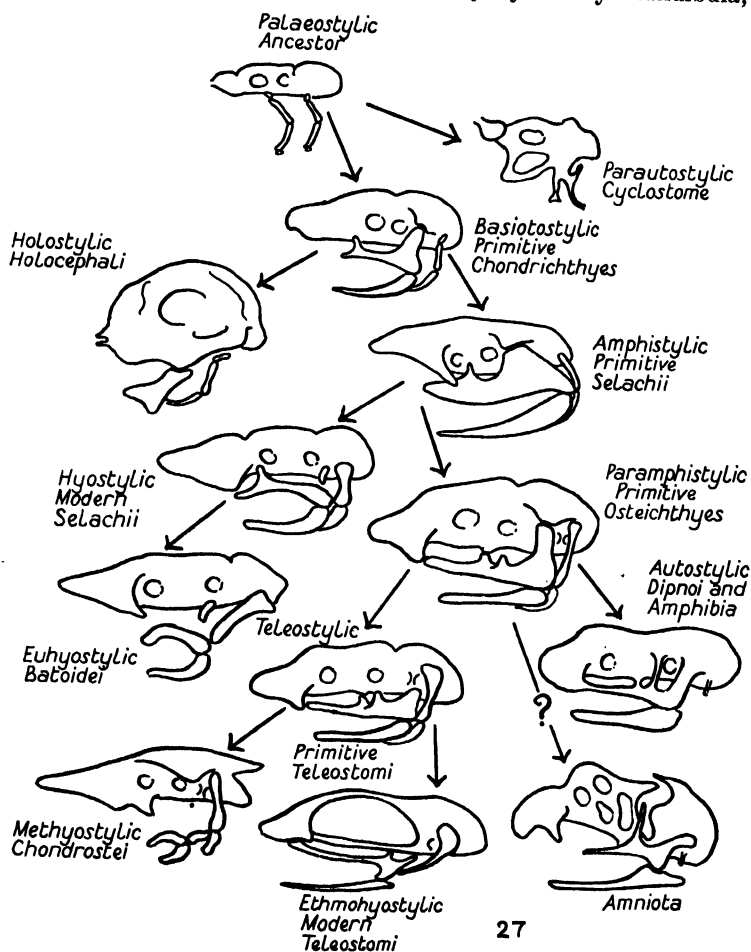
From this type the condition found in the *Holocephali* would have been derived simply by fusion of the arch with the braincase. Gregory's term *Holostylic* is convenient as expressing the completeness of the fusion, and because of its resemblance to *Holocephali*. From the *Basiotostylic* type, the *Amphistylic* ('*Basiotohyostylic*') condition was derived simply by the participation of the hyoid arch in the suspension, with the consequent loss of the pharyngohyal and reduction in size of the spiracle. Here belong the primitive *Selachians*, *Pleuracanthodii*, *Acanthodii*, and *Cladoselachii*. By a subsequent loss of the contact between the otic process and the auditory capsule consequent upon a reduction in the size of the former, or its separation from the palato-quadrate as a spiracular cartilage, the *Hyostylic* forms were derived, as exemplified by most modern *Squaloid Selachians*, e.g. *Squalus* or *Scyllium* ('*Basihyostylic*'), and in exaggerated form by the *Batoids*: the most specialized among these fish ('*Euhyostylic*'). In some *Selachians* contact may also take place between the ethmoid process (foremost extremity of the palato-quadrate cartilage) and the braincase, as in *Scymnus* and *Chlamydoselache*. This scheme, in regarding the *Hyostylic* forms as descended from *Amphistylic* ancestors, agrees with what palaeontological evidence there is, but not with Regan (1906) who considers that the families of *Selachians* without an otic process are more primitive than those which possess one.

The condition in the primitive *Osteichthyes* must have been as follows. The otic and basal processes must have been present, suspending the jaws, and the ethmoid process also, for it is almost universal in these forms. The hyomandibula must have been suspensorial, since it is difficult to imagine this condition to have been lost and reacquired. It follows, therefore, that these hypothetical primitive *Osteichthyes* were derived from *Amphistylic* forms simply by establishing the ethmoid articulation. Two additional points should be noticed. One is that in all *Osteichthyes* the hyomandibula articulates with the

auditory capsule dorsal to the vena capitis lateralis instead of ventral to it as in the Selachians. This condition was reached by means of the formation of a jugular canal covered over by a cartilaginous bridge (lateral commissure, de Beer, 1926) across which the head of the hyomandibula was able to move up to its more dorsal position (Stensiø, 1925). The other point is that the ascending process must have made its appearance at about this time, for not only is it found in the Dipnoi but it is also foreshadowed in the most primitive Teleostomes: the Osteolepidoti (Watson, 1926). The primitive Osteichthyes may therefore be termed Paramphistylic to emphasize the fact that their suspension differs from that of their Amphistylic ancestors in the relations of their hyomandibula. These forms must be regarded as the common ancestors of Osteolepidoti, Palaeoniscids, and other Teleostomes, Dipnoi, and Tetrapoda (Watson, 1925 and 1926). The Dipnoi lost the suspensorial function of the hyomandibula, and effected fusion between the basal, ascending, and otic processes and the brain-case, resulting in the Autostylic type. The Amphibia are similar, with the addition of a fused ethmoid process in many forms. But since in most Amniota the palato-quadrates is not fused with the brain-case, the question arises as to whether the primitive Tetrapoda were not Paramphistylic, in which case the Autostylic condition of Dipnoi and modern Amphibia might have been independently acquired. This is the opinion of Luther (1914), and it agrees with Watson's (1926) demonstration that living Dipnoi and Amphibia have become greatly specialized along convergent independent lines.

The Teleostomes diverged from the Paramphistylic type simply by losing the contact between the otic process and the auditory capsule, and preserving the ethmoid, basal, and hyomandibular suspensions. Such a condition, found in Osteolepidoti, Palaeoniscoidei, and *Lepidosteus*, may be termed Teleostylic ('Ethmobasihyostylic'), characteristic of Teleostomes. A further stage in the reduction of the suspensions is shown by *Polypterus*, *Amia*, and the Teleostei, in which the contact between the basal process and the brain-case is also lost ('Ethmohyostylic').

There remain the Acipenseroides to be considered. These forms have a suspension effected solely by the hyomandibula,



TEXT-FIG. 27.

Diagram to illustrate the probable phylogeny of the types of suspension of the jaws.

and Sewertzoff (1926 B and 1928) is inclined to derive them directly from the Hyostylic Selachians. But not only have

Traquair (1887), Smith Woodward (1898), Stensiø (1921), and Watson (1925) shown that the palaeontological evidence points to the Acipenseroidei having been derived from Palaeoniscoidei (and therefore from Paramphistylis forms), but the hyomandibula of Acipenseroidei articulates with the auditory capsule dorsal to the jugular canal (as in all Teleostomes), and thereby differs from the condition in the Hyostylic Selachians. Gregory's term *Methyostylic* may therefore be appropriately used to denote the fact that the suspension in Acipenseroidei is effected by means of the hyomandibula while drawing attention to the fact that this condition is not the same as the Hyostyly of Selachians.

Expressed in tabular form, this scheme is as follows:

<i>Type.</i>	<i>Suspension effected by.</i>	<i>Term.</i>
Ancestral	Dorsal end of arch = basal process, attachment loose	Palaeostylic
Petromyzon	Dorsal end of arch = basal process, fused	Parautostylic
Primitive Chondrichthyes	Basal and otic processes, attachment loose	Basiotostylic
Holocephali	Basal and otic processes, fused	Holostylic
Primitive Selachii	Basal and otic processes and hyomandibula, attachment loose, hyomandibula ventral to jugular vein	Amphistylic (Basiotohyostylic)
Squaloidei	Basal process and hyomandibula, attachment loose	Hyostylic (Basihyostylic)
Batoidei	Hyomandibula	Hyostylic (Euhyostylic)
Primitive Osteichthyes	Ethmoid, basal, and otic processes, and hyomandibula dorsal to jugular vein, ascending process small, attachment loose	Paramphistylic
Dipnoi and Amphibia	Ethmoid, ascending, basal, and otic processes, fused	Autostylic
Primitive Teleostomes	Ethmoid, and basal processes and hyomandibula, otic process reduced, attachment loose	Teleostylic (Ethmobasihyostylic)
Teleostei	Ethmoid process and hyomandibula basal and otic processes reduced, attachment loose	Teleostylic (Ethmohyostylic)
Acipenseroidei	Hyomandibula (dorsal to jugular vein) attachment loose	Methyostylic

THE PROBLEM OF THE ACROCHORDAL.

The skulls of nearly all vertebrates are characterized by the possession of a pituitary fossa which is bounded posteriorly by a more or less pronounced ridge known as the dorsum sellae. In the development of the various forms this ridge has been called the crista sellaris, acrochordal, and prootic bridge, and where it is well developed it projects up into the plica encephali ventralis. Behind the dorsum sellae it is very common to find a vacuity between it and the parachordal cartilages. This vacuity, which is traversed by the notochord at least in early stages, is known as the fenestra basicranialis. In those forms which possess a pila antotica the dorsum sellae is typically a direct median continuation of the line of the pila antotica. In 1926 I treated the acrochordal, crista sellaris, and prootic bridge as homologous structures, and the fenestra basicranialis as homologous right through the vertebrate series.

This view, with which Allis was at one time in agreement, is now opposed by him in a recent paper (1928). Allis distinguishes two separate transverse cartilaginous structures at the front of the parachordal plate. One of these he calls the commissura acrochordalis, which he regards as situated in the region of the premandibular segment, and in front of the true parachordals. The other is his commissura transversa, which is the anterior edge of the parachordals proper, and which he regards as situated in the mandibular segment. Further, Allis concludes that the vacuity in the basal plate commonly known as the fenestra basicranialis may be of two kinds: (i) a fenestra prootica medialis, between the commissura acrochordalis and the commissura transversa and according to him to be found in *Squalus*, *Acipenser*, *Polypterus*, *Amia*, and *Lepidosteus*; (ii) a fenestra mesotica medialis, behind the commissura transversa, and according to him to be found in *Salmo*, *Amiurus*, *Syngnathus*, and *Lacerta*.

Allis admits that both these fenestrae are not described in any one fish, and, indeed, it would seem that little evidence short of this could be really adequate to establish the truth of his contention. In the first place, attention must be called to what

is surely a slip on Allis's part on p. 131 of his paper, where he says that 'the fenestra basicranialis is . . . simply an opening between the trabeculo-polar bars which leads directly into the primary extracranial subpituitary space'. What Allis is here describing is the fenestra hypophyseos, and the term fenestra basicranialis must be restricted to the vacuity or vacuities in the basal plate (i.e. the fenestrae prootica medialis and mesotica medialis, if they are not identical).

Allis's view is open to attack along more than one line. He would regard the crista sellaris of *Lacerta* as not homologous with the acrochordal of *Squalus*, and yet the similarity in the relations of these structures to the notochord and to the pilae antoticae, as shown by van Wijhe (1922), Gaupp (1900), and myself (1930 B), is such as to make it difficult to doubt their homology. Then again, Allis would deny the homology between the fenestra situated behind the so-called prootic bridge of *Amia* and *Lepidosteus* on the one hand and that of *Salmo* on the other. But his reasons for doing so would seem to be insufficient. There may be slight topographical variations between the fenestrae in question in these animals, which are hard to control owing to the absence of a pila antotica in *Salmo* and *Amia* and its (perhaps doubtful) presence in *Lepidosteus*, with which to check the position of the prootic bridge. But their morphological similarity argues strongly in favour of their homology. It would indeed be odd if, in forms as closely allied as are *Amia* and *Salmo*, structures, as similar as the basicranial fenestrae in these two forms are, were of different nature.

The relations to the pilae antoticae of the acrochordal of Selachians and birds, and of the crista sellaris of reptiles, are identical with those of the dorsum sellae of mammals (man, rabbit) and of the acrochordal cartilage of amphibians and Dipnoi. The pilae antoticae in the mammals are of course represented by the posterior clinoid processes. The conclusion is, therefore, that the transverse ridge forming the dorsum sellae is homologous throughout the Gnathostome series, be it called acrochordal, crista sellaris, dorsum ephippii, or prootic bridge. Consequently, the vacuity in the basal or parachordal

plate immediately behind the dorsum sellae, the fenestra basiscranialis, must also be homologous throughout.

There are considerable differences in the manner in which the dorsum sellae arises in the different groups of vertebrates, but they cannot invalidate the conclusions arrived at above. In *Ceratodus* and Amphibians the dorsum sellae is better defined as the hind border of the hypophysial fenestra, because the pituitary fossa is not well marked, and it is simply the anterior edge of the parachordal plate, and appears early. In *Acipenser* the so-called dorsum ephippii is likewise formed from the anterior edge of the parachordals according to Sewertzoff (1928), and from my observations it would seem to extend farther up the plica encephali ventralis at later stages of development. In birds the acrochordal cartilage is the first element of the chondrocranium to appear, as Sonies (1907) showed and I can confirm. Among mammals the dorsum sellae arises in the rabbit at a late stage according to Voit (1909), as a transverse bar of cartilage connecting the two posterior clinoid processes, which themselves represent the pilae antoticae. In *Lepidosteus*, *Amia* (de Beer, 1926), and *Salmo* (de Beer, 1927) the prootic bridge arises late as a median plate of cartilage between the anterior ends of the parachordals.

In *Lacerta* (de Beer, 1930b) the crista sellaris arises late from paired cartilaginous extensions towards the middle line from the foremost points of the parachordals. In Selachians the dorsum sellae arises late and has a complicated origin. In *Scyllium*, as described in this paper, there is formed a post-pituitary commissure and a precarotid commissure, between the polar cartilages, beneath the notochord, and in the hind region of the hypophysial fenestra. Later, the dorsum sellae, in the form of paired processes directed towards the notochord from the foremost points of the parachordals, forms the anterior edge to the parachordal plate. The conditions in *Squalus* as described by van Wijhe (1922) are probably, though not quite, identical. He describes a cartilage which has the relations of that which is here called the precarotid commissure, and later there are formed paired processes which he says come from the

polar cartilages, and therefore correspond with what has here been called the postpituitary commissure.

The basicranial fenestra has now been observed in all the main groups of the Gnathostomes except the Dipnoi and the Anura. In Urodela it appears to arise by absorption of the cartilage of the parachordals, but in all the remainder the basicranial fenestra is an area of delayed chondrification.

There remains the question as to whether the dorsum sellae is to be regarded as a part of the true cranial floor. There is no doubt that this question must be answered with regard to the position of the true wall of the brain-case as indicated by the position of the dura mater, and not with regard to the cartilage of a dried skull. Therefore, although the dorsum sellae does project up into the cavity of such a dry skull, since it is formed in intimate association with the dura mater, Allis must be right in claiming the cartilage of the dorsum sellae as part of the true skull-floor, and I wrong in denying it (1926).

SUMMARY.

1. The development of the skull of *Scyllium canicula* has been studied from the first appearance of cartilage, through thirteen stages, up to the point at which the main features of the adult skull have been acquired.

2. The parachordals are the first elements to chondrify, and evidence is presented confirming Goodrich's observations concerning the visible traces of metameric segmentation of the metotic region of the parachordal.

3. The auditory capsule chondrifies from the first in continuity with the parachordal, to which it is attached by the anterior basicapsular commissure.

4. The polar cartilages have not been found separate, but they appear as nodules of cartilage attached to the under surface of the anterior ends of the parachordals.

5. The orbital cartilage becomes attached to the parachordal by means of the pila antotica, and to the trabecula at the base of the lamina orbitonasalis by means of the preoptic root.

6. The hind wall of the pituitary fossa is formed in a complex manner, from a postpituitary commissure between the polar

cartilages, and a pair of inwardly directed processes from the foremost ends of the parachordals forming the dorsum sellae. There is also a precarotid commissure, enclosing the carotid arteries in a foramen between itself and the postpituitary commissure.

7. The basicranial fenestra has been demonstrated.

8. Arguments are given for rejecting Allis's view that the so-called basicranial fenestrae throughout the craniates are not homologous.

9. Attention is called to the vacuity in the median wall of the auditory capsule through which the posterior canal bulges, and to the fact that this vacuity is not to be confused with the foramen endolymphaticum.

10. The relations of the glossopharyngeal nerve are described, and it is shown that its apparent passage through the cavity of the auditory capsule is to be ascribed to the fact that the lamina hypotica of the parachordal acts as a false floor to the auditory capsule, the true floor of which is in this region unchondrified.

11. The problem of the relations of the jaws to the brain-case is reviewed in the light of recent investigations, and a reasoned classification is attempted.

12. It is noticed that chondrification is delayed in embryonic material collected from Naples, as compared with material of similar size and degree of development obtained from Plymouth.

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EXPLANATION OF PLATES 32 TO 37.

All figures are of *Scyllium canicula*.

PLATE 32.

Fig. 1.—Left side view of stage 1 (24 mm., Plymouth).

Fig. 2.—Left side view of stage 2 (25 mm.).

Fig. 3.—Dorsal view of stage 3 (28 mm., Naples).

Fig. 4.—Dorsal view of stage 4 (29 mm., Naples).

Fig. 5.—Dorsal view, and

Fig. 6.—Left side view of stage 5 (29½ mm., Naples).

Fig. 7.—Dorsal view, and

Fig. 8.—Left side view of stage 6 (30 mm., Naples).

PLATE 33.

Fig. 9.—Dorsal view, and

Fig. 10.—Left side view of stage 7 (30 mm., Plymouth).

Fig. 11.—Dorsal view, and

Fig. 12.—Left side view of stage 8 (34 mm., Plymouth).

PLATE 34.

Fig. 13.—Dorsal view, and

Fig. 14.—Left side view of stage 9 (35 mm., Plymouth).

Fig. 15.—Dorsal view, and

Fig. 16.—Left side view of stage 10 (36 mm., Plymouth).

PLATE 35.

Fig. 17.—Ventral view of nasal capsule and jaws of stage 10.

Fig. 18.—View from left side and behind of stage 11 (36 mm., Naples).

PLATE 36.

Fig. 19.—Dorsal view, and

Fig. 20.—Left side view of stage 12 (37 mm., Plymouth)

Fig. 26.—Anterior face of left posterior portion of auditory capsule of adult, seen looking back from in front.

Fig. 27.—Anterior face of left posterior portion of auditory capsule of adult, cut surface farther anterior to that of fig. 26, seen looking back from in front.

Fig. 28.—Posterior view of left auditory capsule of adult.

PLATE 37.

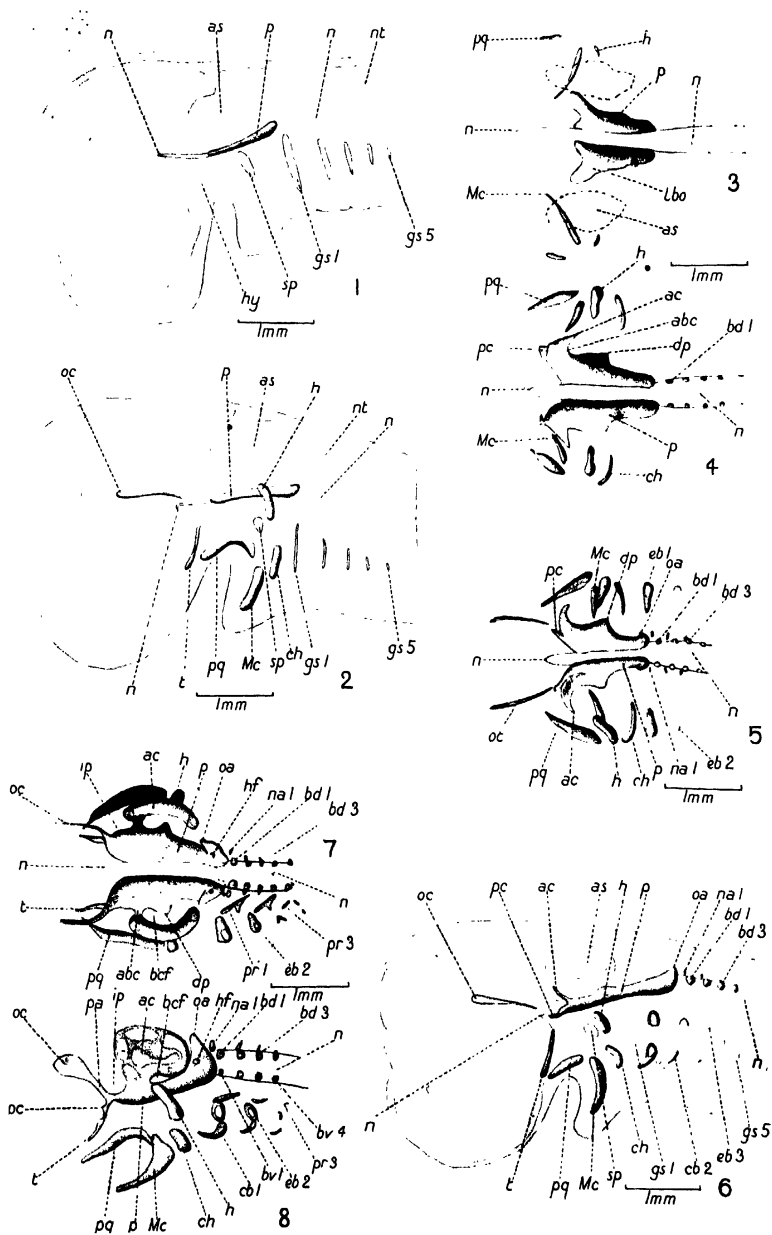
Fig. 21.—Dorsal view, and

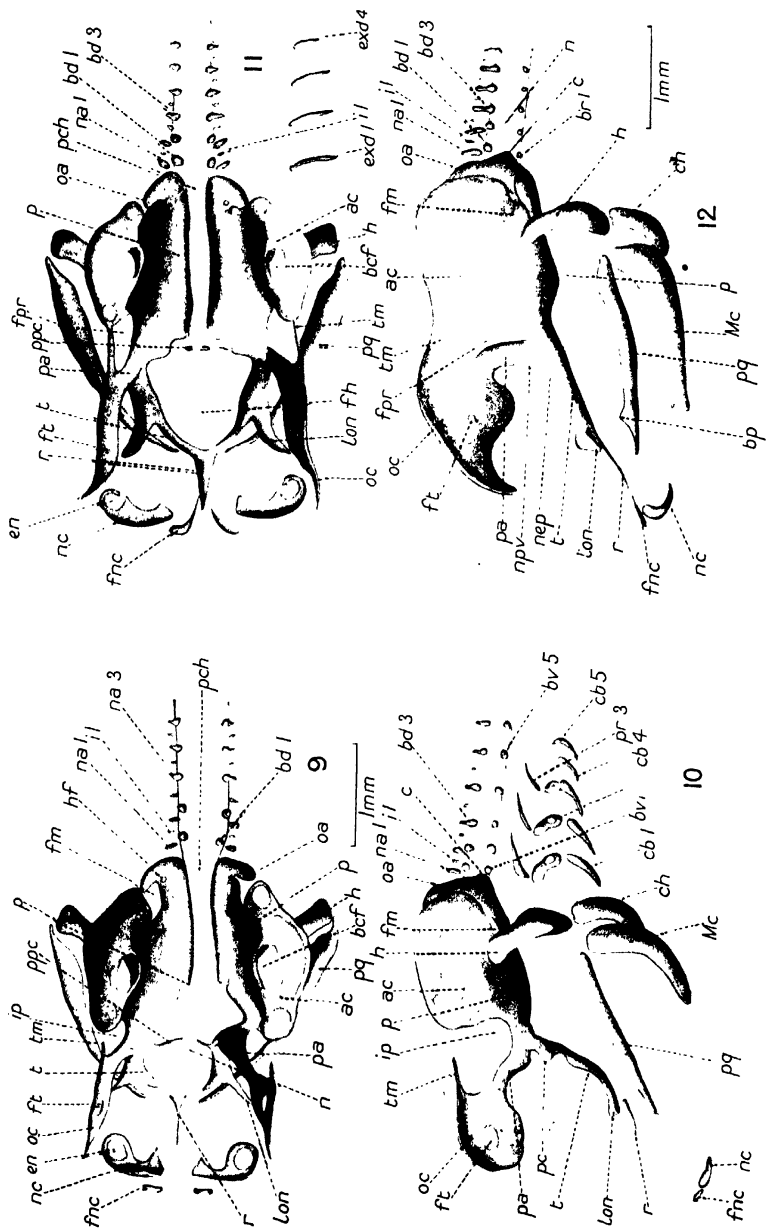
Fig. 22.—Left side view of stage 13 (45 mm., Naples).

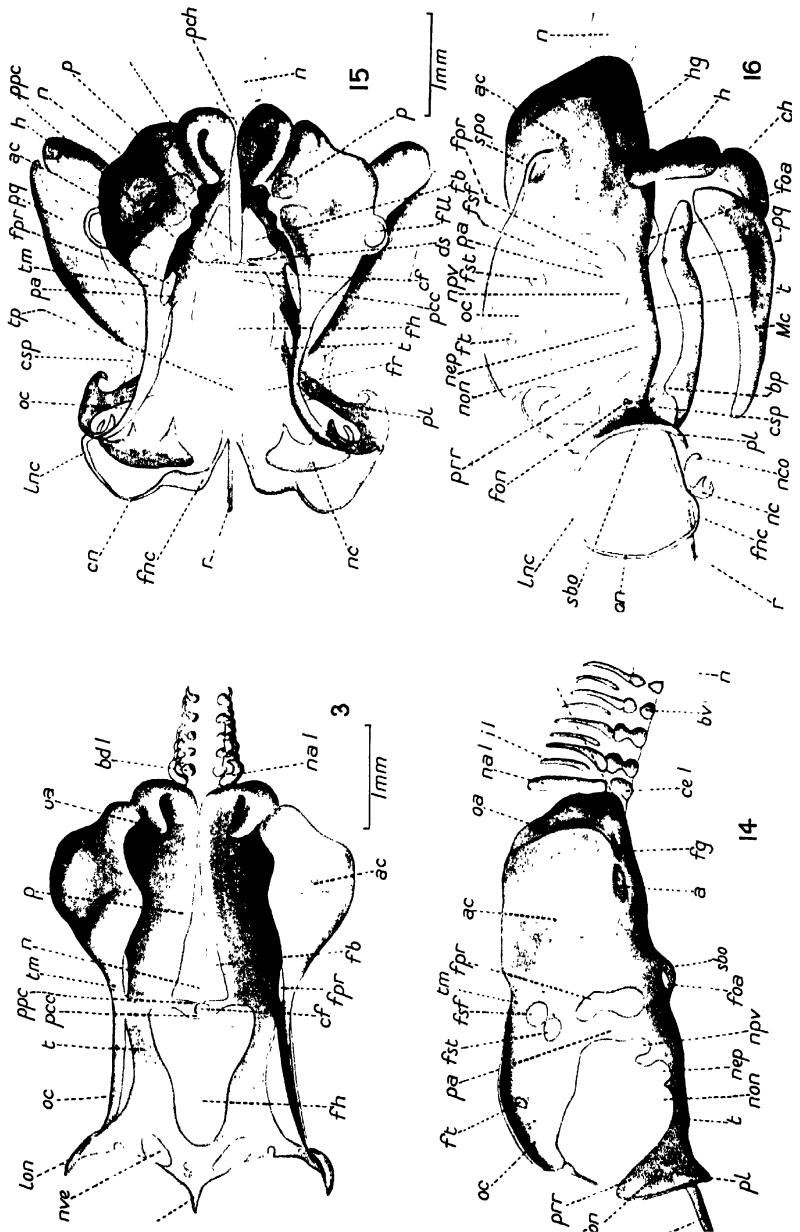
Fig. 23.—Ventral view of nasal capsule and visceral-arch skeleton of stage 13.

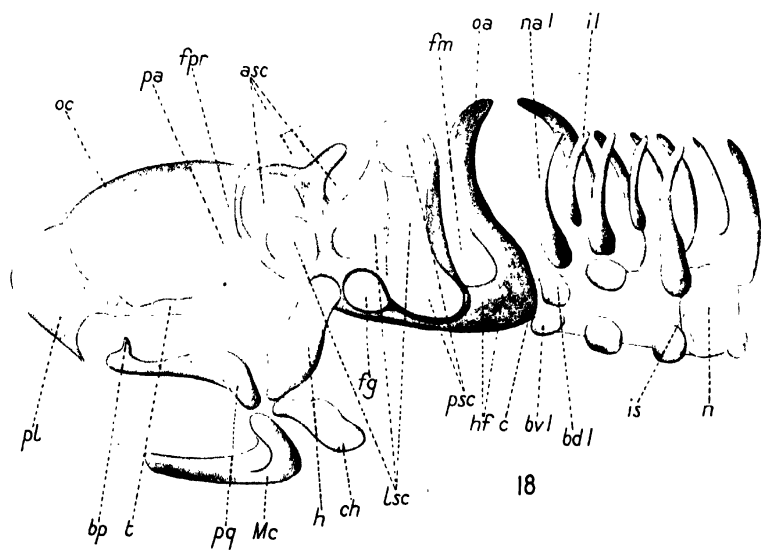
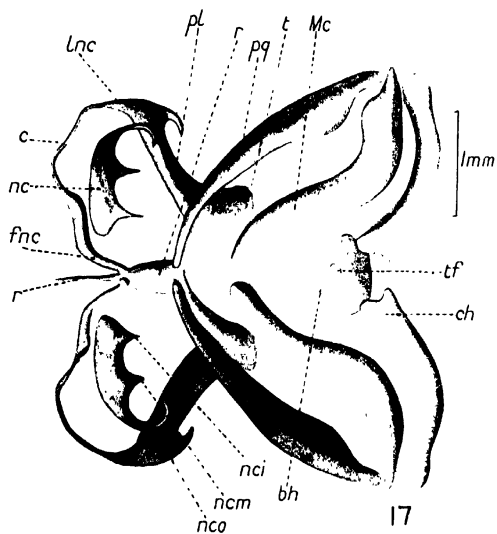
Fig. 24.—Median view of the left side of stage 13.

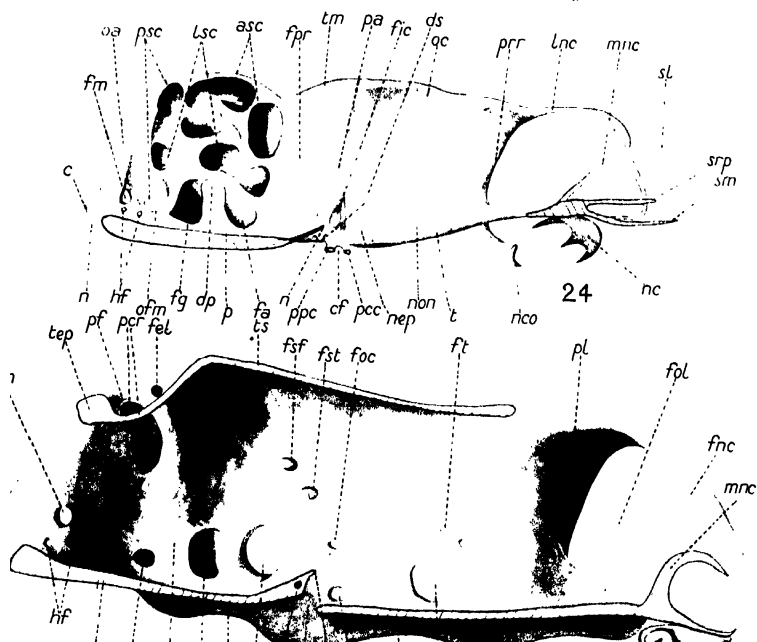
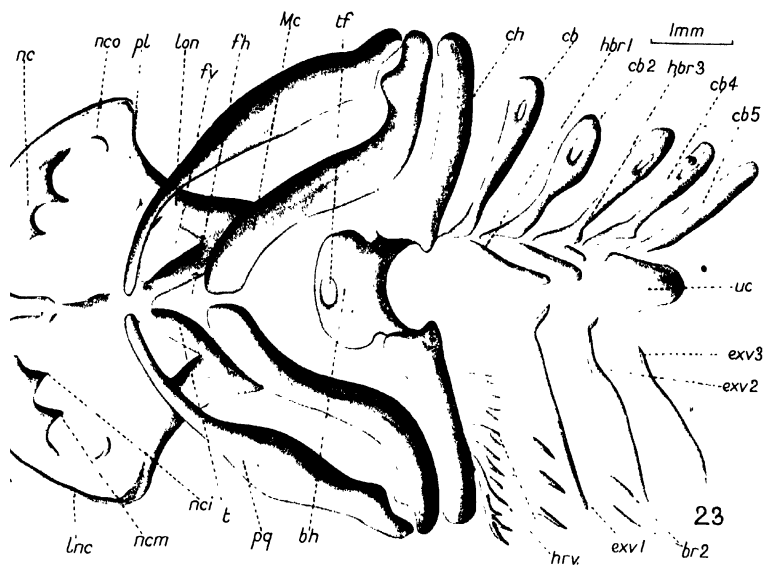
Fig. 25.—Median view of the left side of adult.



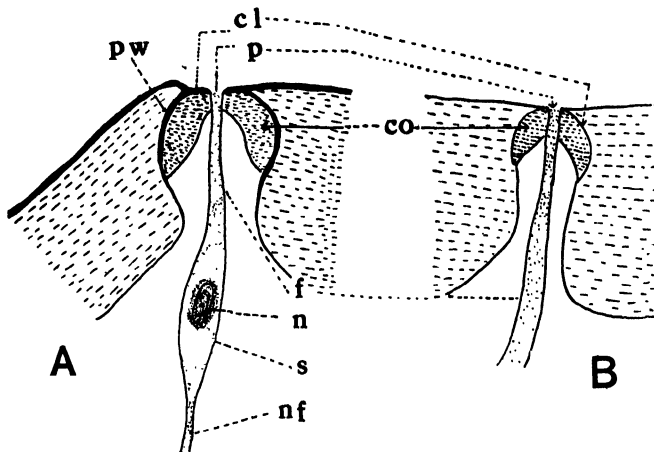








and are consequently freely exposed to the outside air. It is for this reason that he gives the name of olfactory pores to these campaniform sensillae. A number of competent investigators, notably Sihler (1924), Lehr (1923), Hochreuter (1923), and others, have studied similar organs among other insects, but none has been able to detect the peculiar histological



TEXT-FIG. 1.

Histology of 'olfactory pores' according to MacIndoo. *A*, section from leg. *B*, from wing. *cl*, cuticular layer; *co*, chitinous cone; *p*, pore aperture; *pw*, pore-wall.

features described by MacIndoo. So far as the present writer is aware, no studies have been made on the so-called olfactory pores of the honey-bee since MacIndoo's paper. It therefore appeared desirable to reinvestigate these organs in that same insect, using MacIndoo's and other methods of technique. The work was carried out at the Rothamsted Experimental Station at the suggestion of Dr. A. D. Imms, F.R.S., whose unfailing interest and advice I wish to acknowledge.

According to MacIndoo the structure of an olfactory pore from the base of the wing is as follows (vide Text-fig. 1 *A*).

A typical olfactory pore of the honey-bee is an inverted flask in which the bottom of the flask forms the external covering or chitinous layer of the pore (Text-fig. 1 *A*, *cl*). This layer contains

the pore aperture, *p*. The chitinous cone, *co*, is not separated from the pore-wall, *pw*, but it is evidently somewhat different in composition from the surrounding chitin, since it stains less deeply with iron haematoxylin and eosin or in safranin and gentian violet. The sense-cell, *s*, is bipolar, long, slender, and comparatively large. The sense-fibre, *f*, of this cell runs into the hollow of the cone, pierces the bottom of the cone, and enters the lowest portion of the transparent pore aperture. The figure accompanying the above description is actually from the leg of the bee. It is also the most complete that is given and, although those figured by MacIndoo from the wing (Text-fig. 1 B), are less detailed and more schematic, the structure is the same. MacIndoo adds that, since the pore apertures are so small, only occasionally does the microtome knife pass through the lowest part of the aperture. On account of this it is difficult to find a sense-fibre running into the aperture, but when several sections are critically studied it is possible to see several such connexions. He does not deal with the accompanying hypodermal cells.

The distribution and number of the 'pores' are fully described in all three types of bees. For example, he described 21 groups on the worker bee—the first 5 on the wing-bases, 6–18 on the legs, and 8 on the sting—totalling on the average 2,268 'pores' for each worker. On the queen he found 1,860, on the drone 2,604. The pores on the legs present a rather different appearance in surface view from those of the wing.

II. METHODS AND TECHNIQUE.

For the purposes of this investigation the so-called olfactory pores of the wing-bases were selected for study. Both adult and pupal wings were used, the latter being derived from pupae about sixteen and more days old: all were those of worker bees.

In order to investigate the purely cuticular parts of these organs, bases of the wings were detached from the body and placed in a 10 per cent. solution of potassium hydroxide. After washing in distilled water they were stained with carbol fuchsin. Corresponding portions of the wings of other specimens were merely decolorized by treatment with chlorine gas. These were

either left unstained and mounted in 15 per cent. potassium acetate, as recommended by MacIndoo, or stained in toto and mounted in euparal or in Canada balsam.

For the purpose of studying the complete structure of these organs it is obviously necessary to resort to section cutting. Various methods were tried, including the complicated paraffin wax and celloidin technique described by MacIndoo (1926). Simple embedding in paraffin wax of 60° C. melting-point for adult material, and of 54–6° C. for the pupal wings, gave adequate and more certain results.

A number of fixatives were used, including the Carnoy-Lebrun mixture, Sansom's modification of Carnoy's fluid, Gilson's fluid, Worcester's fluid, Bouin's fluid, Schwabe's formula as given by Sihler (1924), and Henning's fixative for chitinous objects. Of these the first two and the last were found most suitable. Good sections were obtained of material fixed in Henning's fluid, the cutting being notably facilitated, and there was no evidence of poor preservation, as mentioned by Hochreuter.

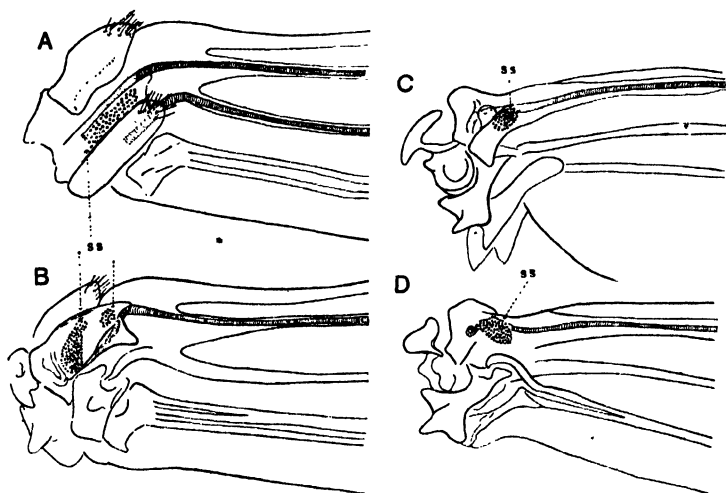
For purposes of staining the usual haematoxylin were all used, including Heidenhain's iron-haematoxylin, Ehrlich's, and Delafield's. Among other stains used were gentian violet and safranin, Mann's methyl-blue-eosin, Mallory's phosphotungstic haematoxylin, and Mallory's phosphomolybdic haematoxylin. In the latter case the formula used was that given by Bolles Lee, and it was applied both with and without mordanting in copper sulphate. From among these various methods the best general results were obtained with Mann's methyl-blue-eosin. For the finer details of structure iron haematoxylin and the two stains of Mallory gave the best differentiation.

Several types of silver impregnation were tried, but the only method that gave moderately successful results was Boeke's modification of Bielchowsky's silver and silver oxide impregnations. A few trials were made with methylene blue both *intra vitam* and *post mortem*, but, owing to the fact that the stain has to penetrate between the two layers of wing membrane, and because it is not possible to watch its progress through the brown chitin, neither method gave good results.

III. THE STRUCTURE OF THE 'OLFACTORY PORES'.

(a) The Cuticular Parts.

Text-fig. 2 shows the arrangement and position of these organs on the wing-bases. On the dorsal surface of the front wing there is a single group; on the ventral surface there is one large group and two groups of smaller size; one of the smaller groups lies in a plane at right angles to the others. The hind wing possesses



TEXT-FIG. 2.

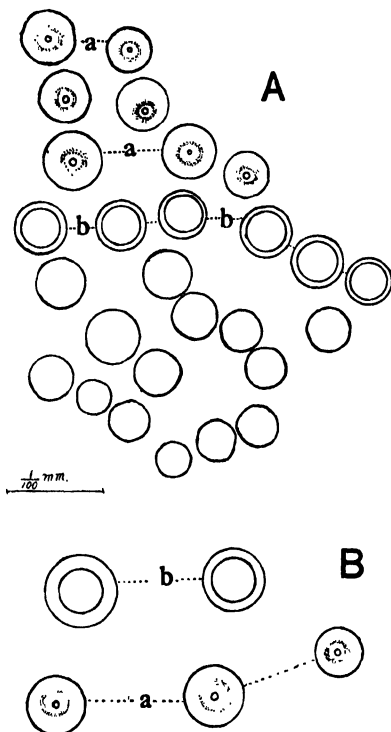
Positions of campaniform sensillae on wing-bases in honey-bee.

A and *C* dorsal views, *B* and *D* ventral views of front and hind wings respectively. *ss*, sense-organs. $\times 40$.

a single group on each surface. It will be seen from Text-fig. 3 that there is a variation in the diameter of the individual organs.

Little of their structure can be made out from surface examination alone. When the ventral sensillae of a wing, which has been bleached and stained, are examined in surface view under a high power, all that is visible is a small central circular spot surrounded by a ring, which is differentiated from the surrounding chitin by its deeper staining properties (Text-fig. 3 *A*, *a*). The central spot is presumably the 'pore aperture' of

MacIndoo, but in order to understand its significance reference must be made to fig. 8, Pl. 39, which shows the sensillae in section. The cuticle in this region is excavated to form a some-



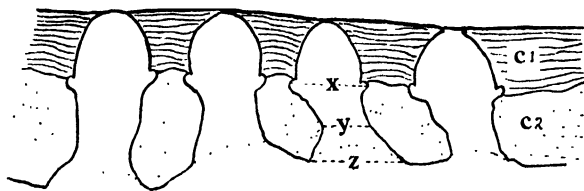
TEXT-FIG. 3.

Surface view of a group of campaniform sensillae from *A* the ventral and *B* the dorsal surface of front wing. *a-a* before, *b-b* after treatment in caustic potash.

what flask-shaped cavity, which is lined with material staining more deeply than the surrounding chitin. This lining substance (*tc*) is the 'Polstermasse' of many German writers and the 'chitinous cone' of MacIndoo; it is here named the terminal cap. It embraces the highly refractive scolopala (*sa*) or termination of the sense-fibre. It is probable, therefore, that the so-called 'pore aperture' of MacIndoo is nothing more than the

refractive apex of the scolopala. The surrounding ring is merely an optical section of the inner lining of the cavity mentioned.

This opinion is confirmed when a specimen (Text-fig. 3 B, b), after treatment with potash and stained in carbol fuchsin, is examined in surface view. The small central spot is no longer visible. By gradually focusing downwards a single ring, which gradually increases to a maximum size, is evident; it then contracts to a smaller diameter, and gradually enlarges again to form the internal opening of the cavity. When microtome sections of material acted on by potash are made (Text-fig. 4) the reason for this difference is apparent. It will be seen that



TEXT-FIG. 4.

Section through ventral sensillae of fore wing after treatment with potash. For explanation of lettering *x*, *y*, *z* see accompanying text. *c1*, *c2*, outer and inner layers of cuticula.

the scolopala and the terminal cap are dissolved away and the series of optical sections observed in surface view above correspond with the sections represented at *x*, *y*, and *z*.

The implication here is that the so-called olfactory pore of MacIndoo is merely the apex of a highly refractive sense-rod; when specimens are suitably treated with potash this is dissolved away and with it the pore-like appearance.

The word suitably is here used advisedly for, if the potash treatment is not too prolonged, it appears from some of the preparations that the scolopala is unaffected, and remains in situ. This would indicate that it has an attachment of some kind to the external layer of the cuticula. If so, then conditions would seem to be similar to those observed by Sihler, in the Acridian, *Gomphocerus rufus*, where he found that the sense-rods of the tactile hairs of the cerci are shed at ecdysis.

The appearance of the dorsal group of sensillae is essentially

similar except that each sensilla appears to lie at the base of a small depression in the chitin (fig. 9, Pl. 39).

(b) Cellular Structure.

Fig. 1, Pl. 38, is a reconstruction of the general appearance, in section, of the dorsal group of sense-organs on the hind wing. It was taken from an adult but incompletely pigmented bee, which had not yet left the pupal cell.

The sense-cell (*s*) appears to be binucleate and bipolar. It narrows proximally into a fibrous strand the identity of which is finally lost in the nerve-bundle, *nb*, which can be traced for some distance towards the thorax. Distally, the cell ends in the sense-fibre, *sf*, ending in a slight swelling, *sa*, which lies centrally within the terminal cap (*tc*) of the cuticular cavity. The apex of this fibre is a highly refractive body and is presumably chitinoid in nature. It completely pierces the terminal cap and lies directly against the very thin cuticular layer that separates it from the outer air. The sense-fibre can be traced proximally as far as the first nucleus, where it appears to be lost in the cytoplasm of the sense-cell. The two nuclei, separated from one another by the median constriction of the cell, present the usual ovoid shape and contain scattered chromatin. The question of the binuclear structure will be referred to again later.

The hypodermis, *h*, appears as a narrow layer surrounding the bases of the sense-cells. In older specimens (fig. 9, Pl. 39) it becomes very attenuated in the parts more remote from the sense-organs. No accessory cells can be definitely associated with the sense-cell but, as is evident from fig. 9, Pl. 39, there are traces of cytoplasmic extensions from the periphery of the sense-cell extending to the terminal cap (*tc*). This figure is from a preparation fixed in Henning's fluid and similar evidence can be found in sections fixed by other methods. .

IV. DEVELOPMENTAL PHASES.

In order to get further evidence as to the nature of the elements forming the sensory complex, late pupal stages were examined. The earliest of these (wherein the eyes begin to show a reddish pigmentation) are shown in fig. 2, Pl. 38, and

fig. 4, Pl. 39 (taken from the dorsal and ventral groups respectively), fig. 2, Pl. 38, being an attempt to reconstruct a number of sections. It will be seen that, although the sense-cell itself is advanced some way in its formation, the actual structure of the cuticular part is in an early stage. The cuticula is, as yet, relatively thin and there is no cavity enclosing the termination of the sense-cell. The latter is short, and comparatively stout, and covered with a dome of deeply staining material (*rtc*) which is the rudiment of the future terminal cap. The rudiment of the sensory fibre is seen at *rf*. Unmodified interstitial hypodermal cells are very evident at this stage. Proximally the sense-cells already appear as binucleate cells gradually tapering off into definitely fibrous processes. These latter are finally collected together into a bundle of nerve-fibres running towards the thorax. In the figure the basement membrane is shown surrounding the whole group of cells but, farther back in the series of sections, it appears to be broken through by the collected strand of fibres. The condition shown in the figure is due to a change in direction of the fibres at the basement membrane; they run parallel to it, i.e. at right angles to their original direction, before collecting together to emerge finally as indicated above. This also accounts for the way in which the fibrous ends of the sense-cells are cut through proximally.

The initial growth stages of the ventral group of sensillae (fig. 4, Pl. 39) are essentially similar, though the cuticle is here very much thinner. Owing to the fact that the sense-cells make their right-angle bend, to run parallel to the flattened plane of the wing, rather sooner (i.e. at the region of the first constriction) than in the case of the dorsal group, they can be followed longitudinally for only a short distance.

A rather later stage of development of the dorsal group of sensillae is shown in fig. 3, Pl. 38. The sense-cells have elongated somewhat, and a certain amount of differentiation has occurred at their distal ends. The lining layer (*tc*) appears as a cap-like termination of the sense-cell, but is actually in contact with the cuticle at only one point. In association with the termination of the sense-cell the following elements may be made out. Firstly, a median fibre-like structure (*f*, in figs. 3

and 6, Pl. 38-9), which can be traced back some distance towards the first nucleus, *nl*, and which is presumably the sense-fibre in course of development. Secondly, surrounding this fibre is differentiated a tract of cytoplasm, *s1*, which itself is flanked on either side by strands of cytoplasm, *s2*, the latter being at least optically distinct from it. These strands stain rather more deeply and are fibrous in appearance. Distally they terminate on the side-walls of the terminal cap—proximally their identity cannot be definitely determined. There are two possibilities: (a) that they are hypodermal cells such as are shown in fig. 3, Pl. 38, as occurring on either side of the region under discussion; or (b) that, as is believed to be the case, they are a result of differentiation within the sense-cell itself. The former theory, involving homologues of the trichogen and hair-membrane cells, would of course be the orthodox one, but it can be scarcely upheld on the evidence here presented and no nuclei can be detected.

A slightly later stage is shown in figs. 5 and 6, Pl. 39. The terminal caps, *tc*, which stain much deeper, are now embedded in the cuticular layer, *c2*, which has been laid down around them. In favourable examples the apex of each cap can be seen to be in contact with the surface layer, *c1*, of the cuticle (fig. 6, Pl. 39). It is evident that the cap forms the future 'lining' of the cavity of the sense-organ, while the rest of the cuticula is laid down by the hypodermal cells. No sign of any pore is to be seen. The triangular process terminating the sense-cell has assumed at its tip a highly refractive appearance, *sa*, which appears to be an early stage in the development of the future scolopala. The surrounding cytoplasm shows the usual deeply staining fibrous nature. The basement membrane, *b*, is shown enclosing, in part, the elongated sense-cells.

From the foregoing evidence it is now possible to come to some conclusion as to the existence of a pore aperture in relation to these sensillae.

MacIndoo, in his summary, says, 'Judging from the structure of these organs it is observed that the cytoplasm in the end of the sense-fibre just beneath the pore aperture is constantly in touch with the outer air'. It is obvious from the foregoing

remarks that if, as it appears to be, the end of the sense-fibre (i.e. the scolopala) is of a refractive chitinoid nature, its cytoplasm cannot be exposed to the outside air. Further, it appears from sections made from material after treatment with potash, that, after the terminal caps have been dissolved away, there is still a thin layer of cuticle separating the cavity of the organ from the outside air. Lastly, no definite evidence of the existence of a pore aperture was found in the surface examinations made. With regard, however, to the identification of the cells forming the sensory complex it is more difficult to reach a decision and the matter is briefly discussed in another section.

V. WING-BASE SENSE-ORGANS IN OTHER INSECTS..

It appears that the sense-organs herein described fall into that class grouped together under the name campaniform sensillae. These have each a single sense-cell and are classified structurally among the tactile organs of the hair-and-peg variety. They have been described from practically all regions of the cuticle in various insects of most orders and have been found in some larvae. The shape of their cuticular parts varies a good deal, as does also the nature of the elements composing the cellular complex.

The most satisfactory information concerning the cellular elements of campaniform organs is given in the paper of Sihler (1924) and includes some very clear figures. The organs are described from cerci of Orthoptera. Their cuticular parts appear to be laid down by a large, well-defined, 'dome forming' or trichogenous cell which is pierced at one side by the sense-fibre from a bipolar nerve-cell. Such a condition seems to differ rather fundamentally from what is apparently the case with the sense-organs figured in the bee, so that the question as to whether the campaniform organs form an homologous group at once arises. It will, therefore, be to the point to review briefly the structure, as described by other observers, of the wing-base organs in other insects.

In the Lepidoptera such work has been done by Freiling and more notably by Vogel (1911). The latter author figures the large elongate sense-cell as the central structure of the sense-

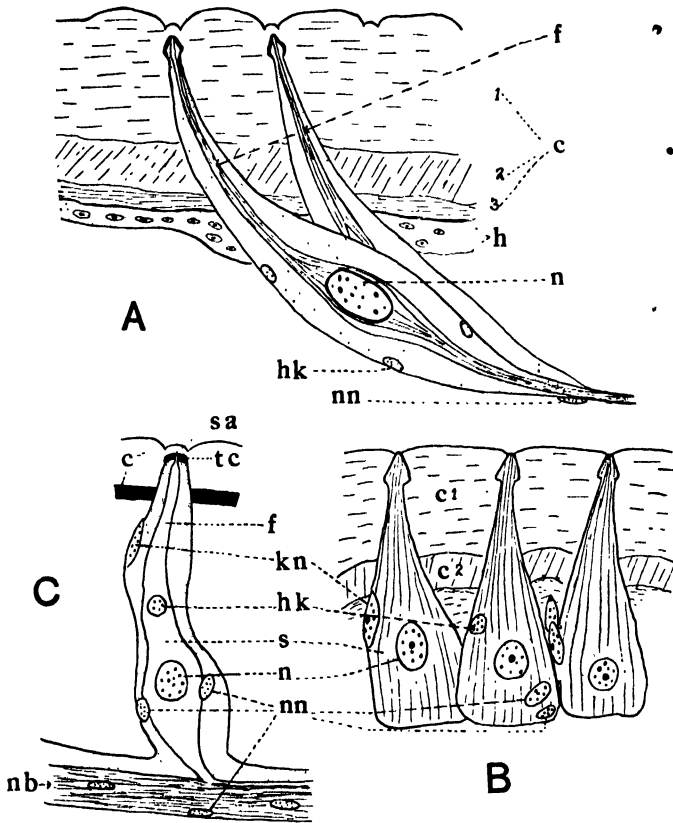
organ, but no dome-forming cells at all comparable with those figured by Sihler are shown. From the presence of two small subhypodermal nuclei, on either side of the sense-fibre at the inner opening of the cuticular cavity, however, he postulates the existence of two auxiliary cells. These he calls the 'Hüllzelle' and the 'Kuppel-' or 'Kappenzelle', but admits there are no cell-boundaries visible. The nerve-cell has a sheathing layer or neurilemma containing a nucleus.

The sense-cells in the bases of the wings and elytra of *Dytiscus marginalis* have been studied by Lehr (1923). This observer also finds supernumary nuclei and associates them with the presence of the 'Kappenzelle' and 'Hüllzelle' of Vogel. His figures (Text-fig. 5 A) of the proximal subcostal group seem to indicate a general structural similarity between these organs and those of the honey-bee.

A free translation of Lehr's description is as follows: Each sense-cell is enclosed by a fairly thick sheet of sheathing cells (Nebenzellen). There can be differentiated therein nuclei of three kinds . . . indicative of three types of cells. First of all there are nuclei of a more elongate shape, with a good deal of scattered chromatin, reminiscent of the neurilemma nuclei of the nerve, but not to be confused with them on account of their considerable size and sharp-pointed ends. They have a constant position and are the most distal of all the nuclei. Perhaps here is seen the ('Kappen-' or 'Kuppelzelle') cap-cell of Vogel . . . Nearby and more proximal to the sense-cell nucleus are nuclei which differ in their more rounded shape. Some of these resemble the hypodermal nuclei; they lie somewhat distal to the sense-cell nucleus and may perhaps be compared with the 'Hüllzellkern' of Vogel. Others, and these lie proximally, must be recognized as similar to the neurilemma nuclei and must be identified with the neurilemma cells of Vogel.

From Text-fig. 5 A it will be seen that, since no cell-boundaries are apparent, each sense-cell is a single elongate multinucleate structure. It is traversed throughout its length by a median fibre which ends distally in the scolopala, divides proximally to enclose the large sense-cell nucleus, and unites again behind it. In the honey-bee this central fibre could not be traced back

farther than the nucleus and, in this respect, agrees with a figure given by Erhardt (1916) from the wing of *Agrion*



TEXT-FIG. 5.

Sections through *A*, the proximal; *B*, the distal subcostal group of the hind wing of *Dytiscus marginalis*: adapted from Lehr. *C*, the schematic constitution of sensory complex which Lehr derives from them. *c1*, 2, and 3, layers of cuticula. *f*, 'Terminal-strang' (sensory fibre); *hk*, 'Hüllzellkern'; *kn*, 'Kuppelzellkern'. For other lettering see Explanation of Plates.

puella. Only one supernumary nucleus is figured in this sense-cell which she calls the 'Hüllzellkern'. In *Chrysopa*

vulgaris, however, the sense-cell is enveloped in a sheath containing two 'Hüllzellkern' and structurally, therefore, she says, it is essentially similar to those described by Freiling, Günther, and Vogel in Lepidoptera. In her figure of an organ from the hind wing of *Locusta cantans* but one accessory nucleus is shown and this lies just beneath the terminal cap itself—actually at the distal end of the cuticular cavity. In the Hymenoptera she gives a single figure from the wing-base of a wasp, *Vespa rufa*. No accessory cells are shown. The letterpress reads, 'In axial section is seen a rounded surface-pit in whose neighbourhood the chitin is slightly raised. Across the pit projects a small cap (Kuppel) through which a fine canal nearly reaches the outside. It stains deeply . . . The attached sense-cells do not differ essentially from those described above; their long spindle shape is particularly emphasized. They are closely packed together and between them are found numerous supporting cells (Stützzellen)'.

A single figure given by her, of the wing-base organs of *Eristalis tenax*, agrees with that from *Vespa rufa*; here, apparently, no sheathing cells and no accessory nuclei occur in association with the sense-cell.

VI. THE CELL ELEMENTS COMPOSING THE SENSE-ORGANS.

The question here raised concerns the number and identity of the cellular components of each sensory unit.

It is generally supposed that the different types of insect sense-organs, with the tactile hairs (*sensilla trichodea*) at one extreme and becoming modified in various ways through the *sensilla chaetica*, *sensilla basiconica*, &c., to the simple campaniform organs at the other extreme, represent a continuous evolutionary series. The tactile hair is presumably the more primitive type and the others must be derived from it. This being so, it follows that evidence of the presence of cells composing the sensory hair complex should be forthcoming throughout the series.

Theoretically three cells are involved.

1. The hair-membrane cell, also called the distal enveloping cell or cap-cell.

2. The trichogenous cell, also termed the basal enveloping cell or the envelope cell.

3. The nerve-cell.

Such cells are indeed described by Schneider (1923) in the hair sense-organs of the cabbage caterpillar (*P. brassicae*). The innervation here, it should be noted, is by a sub-hypodermal nerve-cell whose distal process penetrates the basement membrane and trichogen cell.

Essentially the same structure occurs in the tactile organs of adult insects. As an example, the sensory hairs described by Sihler from the cerci of Orthoptera may be mentioned. Each hair has at its base a large, well-defined, trichogenous cell, which is innervated from the side by the sensory process of an intra-hypodermal nerve-cell.

This condition is obviously different from that occurring in the honey-bee. In the latter insect the main central element of each sensilla is the sense-cell which itself appears to secrete non-cellular parts of the sense-organ and also to give rise to the sensory fibre. There are two possible explanations of these differences. Either there is no homology between these campaniform sensillae and other sensory organs of the tactile hair type, or else the nerve-cell has usurped in some measure the function of, or become intimately fused with, the two accessory cells which, in their turn, have lost their separate identity. Such is presumably the opinion of Vogel and of Lehr. The latter author regards the sensory cell as being surrounded by a syncytial sheath of cytoplasm in whose nuclei he recognizes membrane cell nucleus and trichogen cell nucleus (Kuppelzellkern and Hüllzellkern, Text-figs. 5 A, B, c). Some of Lehr's figures, it may be said, do resemble to some extent those given here (fixed with Henning's fluid), but unfortunately he has not studied developmental stages. In fact the writer is not aware of the existence of any adequate figures of the development of these wing-base sense-organs. It is reasonable to suppose that in the earlier stages, if anywhere, definite evidence of the separate component parts of the sensillae might be obtained. In the earliest pupal stage of the bee examined, however, there appears to be little or no evidence of accessory cells being

involved. It appears, in fact, that a single cell lays down the sensilla and becomes differentiated within itself and that this differentiation, in the case of the sensory fibre, takes place from before backwards—i.e. distally—proximally. It is considered that, in this case, such optical differentiation as is apparent is not sufficient evidence of a sheath of accessory hypodermal cells as has been described by Lehr, for *Dytiscus* (see Text-fig. 5 c). The accessory nuclei occurring in the honey-bee are judged to be those of the ordinary hypodermal cells laying down the cuticula.

As is pointed out in the previous section, Erhardt's figures from the wing-base of *Vespa rufa* show neither cap nor trichogen cells, and a similar absence of these structures appears to occur in the sense-organs of the halteres of some Diptera. The figure given in 'Gli Insetti', p. 684, for example, of a section through the haltere sense-organs of *Tabanus*, is very similar to those figured here from the honey-bee. In both cases, incidentally, the sense-cell is uninucleate, and this raises the question of the binuclearity of the sense-cells in the honey-bee. It may be said at once that this feature is concluded to occur largely as a deduction from the following considerations. At the region of their first constriction the majority of the cells turn through a right angle to run parallel with the basement membrane. They are, therefore, usually cut off short at this region in sections; but, proximally to these cut-off ends, there lie cells beginning distally with a constriction and widening out into a typical cell of the bipolar form. Although these two sets of cells lie in different planes it is believed that one is a continuation of the other. This conclusion is supported by more direct evidence where occasionally, in favourable cases, the continuity is more apparent. It may be added that binuclear sense-cells are figured in 'Gli Insetti', p. 624, from the dorsal region of the antenna of *Sphinx convolvuli*.

VII. ON THE ORIGIN OF THE SENSORY NERVE-FIBRES.

It is the case that a notable difference between the sensory nervous system of vertebrates and invertebrates consists in the position of the sensory neurons. 'In the earthworm we have a

primary sensory neurone with its cell body in the skin and its nerve process ending in ramifications of the neuropile of the segmental ganglia.' This is typical of invertebrates. 'In the vertebrates . . . the primary sensory neurones instead of having their cell bodies in or near the surface have undergone a change in situation . . . so that the cell body is placed . . . close to the central nervous system' (Bayliss, p. 464 et seq.).

It is to be expected, therefore, that the sensory neurons of insects would lie in the hypodermal layer, but this has never been demonstrated. At the same time no one has so far been able to prove their existence in the central ganglia. The position has been well summed up by Snodgrass (1926) and is recapitulated here. He points out that since no sensory neurons have been found in the central ganglia or anywhere associated with them, general opinion follows that of von Rath in insisting that the generative cells of the sensory system must be found in the periphery, i.e. in the ectodermal tissue of the body-wall. Now the only cells so far found in the course of the sensory nerves are the peripheral sense-cells themselves.

The sensory nerve-fibres, when traced outwards, are found to end in cells, which may be multipolar or bipolar and are placed by Zawarzin (1912) into two categories. The first of these is made up of bipolar cells whose distal processes go direct to specific ectodermal sense-organs. The second includes bipolar, or more usually multipolar cells, which give off terminal processes ending in fine branches on the inner surface of the hypodermis. They may also supply the skeletal and abdominal wall-muscles. The cells described in this paper belong to Zawarzin's type 1, and such cells (and indeed those of type 2 as well) are the only ones that occur in the whole course of the sensory nerves. This fact has given rise to the idea that they are the neurons of the sensory fibres. The developmental origin of the sensory cells of type 1 has been studied by many investigators and all agree that they are specialized hypodermal cells, but no one has demonstrated the growth of a nerve axon from these cells. On the other hand, several observers have claimed that a nerve-fibre grows outwards and unites with the sense-cell. The base of the sense-cell, Vogel says, may elongate slightly

towards the nerve, but the connexion with the latter is made in the immediate neighbourhood of the cell. He claims that the innervated cell of an insect sense-organ becomes secondarily a sense-cell by union with a nerve-fibre. On the other hand, his conclusion that there must be found in the deutocerebrum a ganglionic nerve-centre from which antennal sensory cells take origin has never been substantiated.

It is suggested that the neuron of the sensory system connected with the organs herein described is situated in the hypodermis and is in fact the sensory cell itself. The evidence for this is as follows:

1. The proximal elongation of the sense-cells and the consequential pushing-back of the basement membrane by them.
2. The nature of the proximal ends of the sense-cells which narrow down to form fibrous processes which collect together and apparently form the bundle of fibres which can be traced back towards the thorax.
3. The formation of the distal sense-fibre (the fibre of the sense-organ itself) which appears to be differentiated from before, backwards, i.e. distally—proximally.

Against this suggestion it may be urged that a connexion with the sensory nerves from the central nervous system may occur much more proximally. It is conceivable that fibres from an outgrowing nerve may meet and fuse with inward prolongations of the peripheral sense-cells. This is considered to be unlikely in the present case, but only by examination of earlier pupal material than that studied here could the matter be definitely settled.

VIII. ON THE FUNCTION OF THE 'OLFACTORY PORES'.

Hicks, in 1857, was the first to describe examples of these peculiar organs from the legs and wing-bases of insects, and thought that they might have an olfactory function. Since then many workers have studied them, but none except MacIndoo has been able to uphold this view. It is generally considered that they are tactile organs of some kind though, in the case of those occurring on the wing-bases, it is not clear what tactile

function they could perform. Especially is this so when they are almost walled in by folds of chitin so that they cannot be seen in surface view. Lehr describes such conditions from the wing of *Dytiscus*. With regard to wing-base organs themselves, Erhardt points out that they are most numerous on the strong flyers and, occurring as they do on both wings, it would seem reasonable to suppose that they are in some way connected with flight. Presumably some mechanism is necessary to correlate wing vibration with varying air conditions and pressures, but it is difficult to speculate with so little basis of experimental knowledge. Such experimental knowledge, so far as the writer is aware, is limited to the experiments of MacIndoo (for those of Sihler were concerned only with the cercal campaniform organs of Orthoptera) and his results conflict with those of von Frisch (1919). Von Frisch has definitely shown that the olfactory sense resides in the antennae of honey-bees. Two main lines of experiment indicate this. He has shown that, firstly, bees trained to recognize colours still continue to do so after amputation of the antennae; secondly, bees trained to an odour entirely fail to distinguish it after amputation of the antennae. These experiments seem to indicate that the second result is not due to shock following amputation but is caused by the loss of the olfactory organs themselves.

Further, the eight distal segments of the flagellum of the bee's antenna differ from the three proximal segments and the scape, in being supplied with the so-called 'pore plates'. If all of these eight distal joints are cut off then the bee is incapable of recognizing the odour to which it has been trained. If, on the other hand, only one of these distal segments on either antenna is left intact then the bee is still able to respond. When this remaining segment is amputated the bee finally loses this ability. As von Frisch remarks, with reference to the question of shock induced by such experiments, it is difficult to believe that the physiological processes of the bee should be so much more deranged by the loss of all eight distal segments of both antennae than from the loss of eight on one side and seven on the other.

MacIndoo found, however, that the resultant shock invalidated results from experiments involving antennal amputation. The

criterion he used for measuring response was the amount of time taken by the bees to react in some visible way (for example, by vibrating the antennae) to certain essential oils which were placed in close proximity to them. For normal bees the reaction time was found to be on an average 2·8 seconds; when one antenna was cut off this reaction time was doubled; when distal segments 2-8 of the remaining flagellum were removed, the reaction was increased to from 15 to 88 seconds, *pari passu*. This could be taken as direct evidence in favour of the antennae being the organs of olfactory sense—indeed MacIndoo says that at a first glance it does so appear. He disregards it apparently because the behaviour of antennaless bees is generally abnormal and because of the positive reaction (2·9 secs.) and more normal behaviour in the presence of the same stimuli of at least a proportion of those bees which have their antennae intact but coated over with glue.

On the other hand, when the 'olfactory pores' on the wings were removed the reaction time was increased to 27 seconds; when, in addition, the pores on the legs were coated over with certain substances the average reaction time was 40 seconds. This is presumably the chief argument for supposing the olfactory pores to be the main receptors of olfactory stimuli, for the bees in these last experiments had their antennae intact and uncoated.

While one is led to conclude, therefore, from von Frisch's experiments, that the olfactory sense in the honey-bee mainly resides in the antennae, a certain capacity for odour perception is also betrayed by the campaniform organs, according to MacIndoo.

Two points may be mentioned here. The first is that, having regard to MacIndoo's observations with antennaless bees, the difference possibly lies in the interpretation of the results rather than in the results themselves. The second, as has been pointed out by Snodgrass, is that, whereas von Frisch used the milder floral odours under open-air conditions, MacIndoo used powerful smelling and possibly irritant substances under confined conditions. Hence it may well be that, while the antennae are the organs enabling the bee to respond to the normal odour

stimuli encountered during foraging flights, it is capable of detecting the presence of strong smells at close quarters by other means.

IX. SUMMARY.

1. The structure of the campaniform sensillae occurring on the wing-bases of the adult worker bee is described.
2. The essential features in their later developmental phases in the pupa have been followed.
3. The observations described lend no support to those of MacIndoo, that the actual termination of the nerve-fibre is exposed to the outside air.
4. The wing-base organs, as described by other workers in different insects, are discussed, with special reference to the identity of the cellular elements composing these structures.
5. The paper concludes with a short discussion of the position of the sensory neuron and a brief review of the supposed function of the campaniform sensillae.

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XI. EXPLANATION OF PLATES 38 AND 39.

REFERENCE LETTERING.

b, basement membrane; *c1*, *c2*, outer and inner layers of the cuticula; *cc*, cuticular cavity; *f*, sensory fibre; *h*, hypodermis; *n*, nucleus of sense-cell; *nb*, nerve-bundle; *nf*, nerve-fibre; *nn*, nucleus of neurilemma; *rtc*, rudiment of terminal cap; *rf*, rudiment of sensory fibre; *s*, sense-cell; *sa*, scolopala; *tc*, terminal cap.

PLATE 38.

Fig. 1.—Section through dorsal group of sensillae of hind wing of unpigmented adult bee (extracted from cell). Fixed with Henning's fluid; stained in iron haematoxylin. $\times 760$.

Fig. 2.—Section through dorsal group of front wing. Early pupal stage (eyes showing pigmentation). Fixed in Carnoy-Sansom; stained in iron haematoxylin. $\times 760$.

Fig. 3.—Section through dorsal group of front wing; stage slightly later than fig. 2. Fixed in Carnoy-Lebrun; stained in gentian violet and safranin.

PLATE 39.

Fig. 4.—Section through ventral group of front wing; same stage and treatment as fig. 2. $\times 790$.

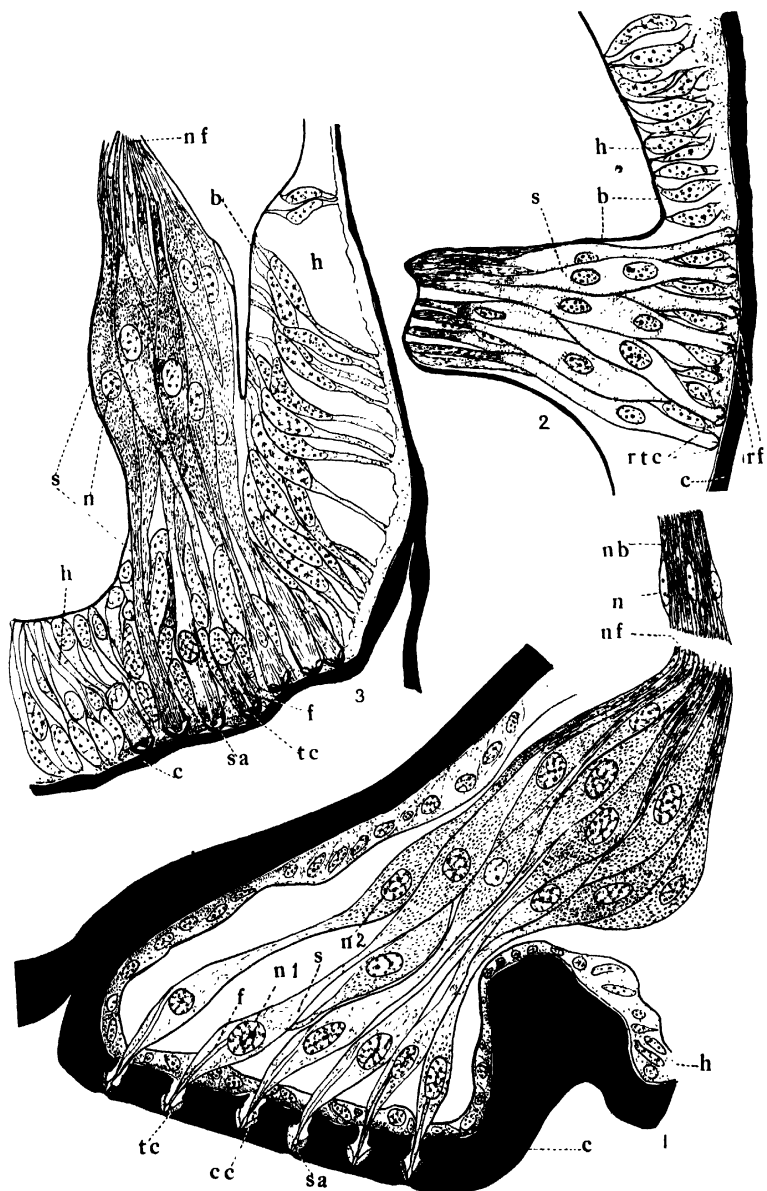
Fig. 5.—Section through dorsal group of fore-wing; later pupal stage. Fixed in Henning's fluid; stained in iron haematoxylin. $\times 730$.

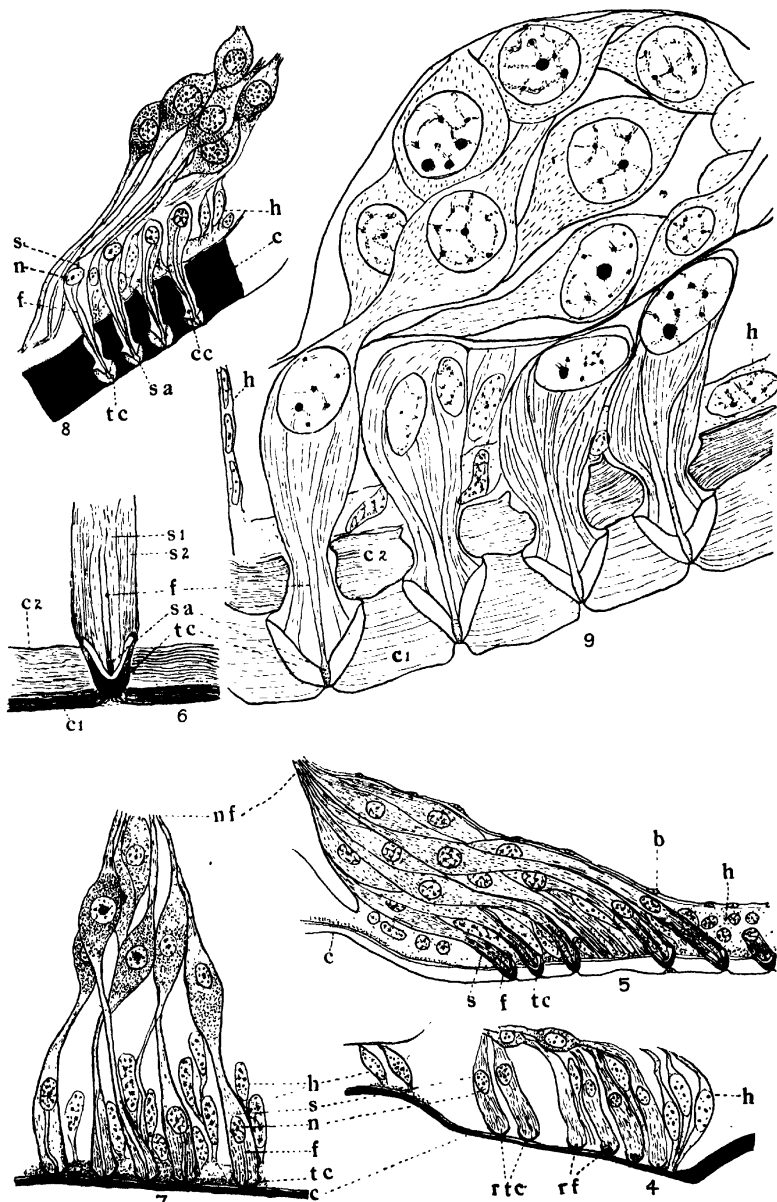
Fig. 6.—Distal part of sense-cell from fig. 5. $\times 1030$.

Fig. 7.—Section through ventral group of front wing, later pupal stage than in fig. 4. Fixed in Carnoy-Sansom, stained in iron haematoxylin-eosin. $\times 790$.

Fig. 8.—Section through ventral group of front wing, late pupal stage. Fixed in Carnoy-Lebrun; stained in phosphotungstic haematoxylin. $\times 730$.

Fig. 9.—Section through dorsal group, hind wing, of adult bee. Fixed in Henning's fluid; stained in Ehrlich's haematoxylin. $\times 2380$.





Studies in the Origin of Yolk.
VI. The Crustacean Cogenesis.

By

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With 31 Text-figures.

INTRODUCTION.

RECENT work from this laboratory has demonstrated that the vacuolar system (vacuome) and the Golgi apparatus are independent cell-components. The eggs of *Rana tigrina* (Nath, 1930*a* and 1931) and the teleostean fishes *Ophiocephalus punctatus* and *Rita rita* (Nath and Nangia, 1931) have turned out to be classical objects for such a demonstration, for not only the mitochondria and the Golgi elements but also the vacuoles can be seen *intra vitam* side by side separately without the aid of neutral red or osmic acid. This is due to the greater density and the larger size of the vacuoles of these three species. In *Ophiocephalus punctatus* the vacuoles begin condensing inside them protein material from a very early stage in oogenesis and actually form the albuminous yolk of the egg, as has been very rightly claimed for *Perca* and *Pygosteus* by Hibbard and Parat (1927 and 1928) and by Hibbard (1928) for *Discoglossus*.

Chemically the vacuoles of the eggs of *Rana*, *Ophiocephalus*, and *Rita* are radically different from the Golgi elements. Whereas the latter consistently go jet-black in either Da Fano or Mann-Kopsch or Kolatchev, and cannot be stained with neutral red, the former do not show the slightest amount of blackening however heavy the impregnations, and

With regard to the technique we desire to emphasize the important fact that our results are based on fresh cover-slip preparations, and the fixed preparations have been used more or less for the purposes of control. The ovaries of the prawn were stained with neutral red and janus green B by keeping the live animals in dilute watery solutions of these vital dyes overnight, thus eliminating any risk of artifacts which may possibly appear when the ovaries are stained after their removal from the animal. During the period of about twelve hours the whole of the alimentary canal and the ovaries are stained pink or green according to the dye used. Ovaries were also osmicated in 2 per cent. osmic acid for short periods and studied directly in a drop of water. This method, which has been extensively used by Nath, is admirable for the study of the lipoidal Golgi apparatus. Absolutely fresh material was also repeatedly studied in a drop of normal saline. Sections were also cut by the usual laboratory technique, using such fixatives as Bouin, Champy, Flemming-without-acetic, Kolatchev, Mann-Kopsch, and Da Fano.

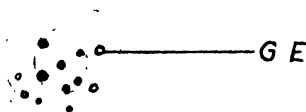
OBSERVATIONS.

Palaemon lamarrei.

(1) Golgi Apparatus and Fatty Yolk.

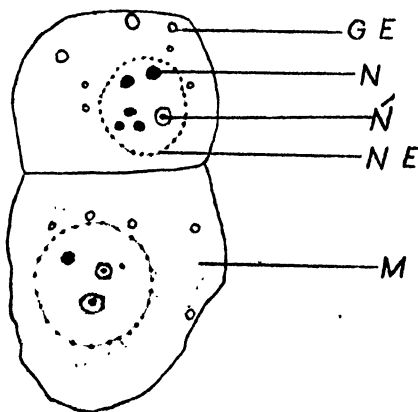
In the youngest oocytes osmication is essential for the demonstration of the Golgi elements, which fail to appear either in absolutely fresh material or in oocytes treated with neutral red. As a rule the oocytes must be immersed in 2 per cent. osmic acid for about twenty-two hours before the Golgi elements can be made to appear. Once, however, they appeared after six hours osmication. In what we consider the youngest oocyte (Text-fig. 1) the Golgi elements appear as vesicles, each showing a blackened rim and a very light brownish interior. The blackening of the Golgi material by osmic acid in twenty-two hours proves that it contains some fat-like substance. In older oocytes (Text-fig. 2) the Golgi elements appear as before. With the further growth of the oocyte they begin to spread out in the cytoplasm (Text-fig. 3). In Text-fig. 4 is represented a fragment of an oocyte measuring about 0.8 mm. The Golgi

elements have more or less uniformly spread out. At the same time not only many of them have grown in size but the interior of the larger ones appears dark brownish whereas the smaller ones still show a light brownish interior like the



TEXT-FIG. 1.

2 per cent. osmic acid for $23\frac{1}{2}$ hours. $\times 355$.



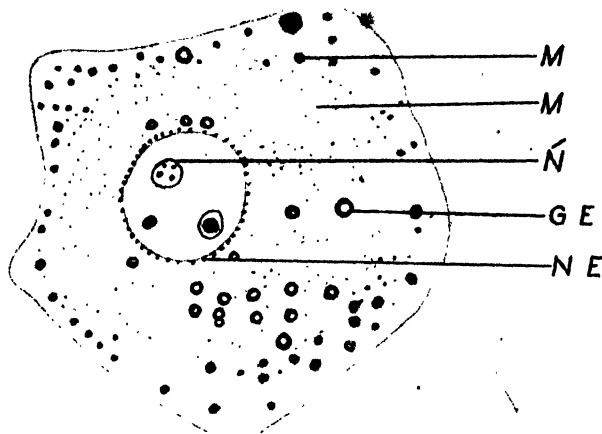
TEXT-FIG. 2.

2 per cent. osmic acid for 22 hours. $\times 355$.

Golgi elements of the younger oocytes. Hereafter it is impossible to study the Golgi elements on account of opacity which results from the sudden appearance of albuminous yolk. The oocytes

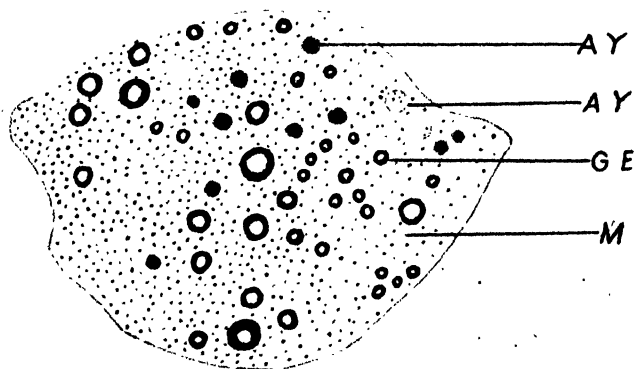
ABBREVIATIONS FOR ALL FIGURES.

AY, albuminous yolk; *GE*, Golgi element, or its product, the fatty yolk; *M*, mitochondria; *N*, nucleolus; *N'*, nucleolus showing internal differentiation; *NE*, nucleolar extrusion. Further explanation of figures will be found in the text. Figs. 1 to 23 are those of *Palaemon*, and figs. 24 to 31 those of *Paratathusa*.



TEXT-FIG. 3.

2 per cent. osmic acid for 24 hours. $\times 355$.



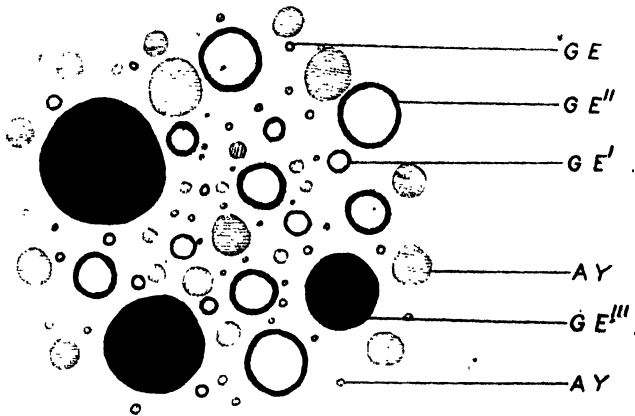
TEXT-FIG. 4.

Fragment of an oocyte measuring 0.3 mm. kept in 2 per cent. osmic acid for 22 hours. $\times 355$.

are therefore ruptured after osmication and their contents are allowed to flow on the slide. In Text-fig. 5 are shown the contents of one of the biggest ovarian oocytes, which had been

osmicated for ten minutes only. The striated spheres represent albuminous yolk which appears whitish. The smallest Golgi elements (*GE*) show a dark rim and a very light brownish centre. The bigger ones (*GE'*) show a dark brownish centre, and the still bigger ones (*GE''*) show a still darker centre. The biggest ones (*GE'''*) appear uniformly black, as their interior and the rim are blackened to the same extent.

From these facts it is to be concluded that the Golgi elements



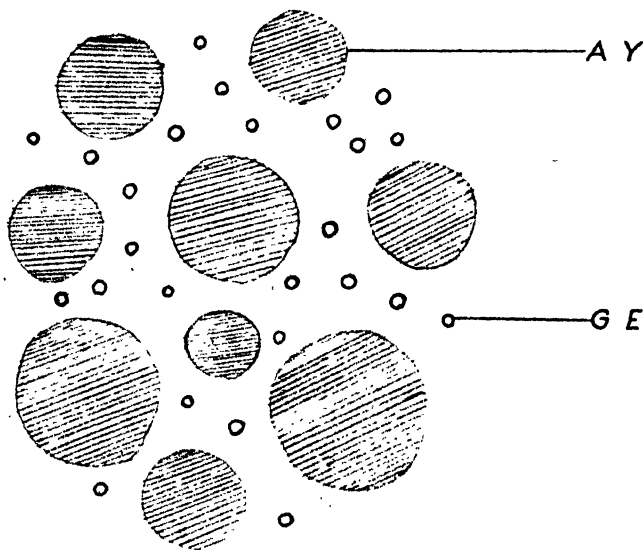
TEXT-FIG. 5.

Contents of a 'ripe' oocyte kept in 2 per cent. osmic acid for 10 minutes. $\times 150$.

not only grow enormously in size but also become more and more fatty. The fully grown Golgi elements may be called the fatty yolk. That it is actually used as nourishment in the course of development is proved by its entire absence from the segmenting eggs (Text-fig. 6).

Fixed preparations confirm the results obtained from the study of the fresh material. We have employed the Kolatchev and the Da Fano techniques for the demonstration of the Golgi apparatus. Although we have succeeded in impregnating the Golgi elements with the latter technique, they appeared as very

much shrunken granules. The former technique, on the other hand, has given us very satisfactory results, the optimum period of incubation in 2 per cent. osmic acid being about four days at 30–35° C. In Text-figs. 7, 8, and 9, which represent an oogonium and two young oocytes, the Golgi elements appear as jet-black granules and not as vesicles. A comparison of these

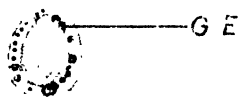


TEXT-FIG. 6.

Contents of a segmenting egg kept in 2 per cent.
osmic acid for 10 minutes. $\times 200$.

figures with Text-figs. 1 and 2 will show that the number of the Golgi elements is much larger in the former. This discrepancy is undoubtedly due to the fact that the Kolatchev technique, as the result of prolonged osmication at a higher temperature, has brought to view not only the granular type of the Golgi element but has also made the tiny vesicles to appear as solid bodies. When, however, the Golgi elements begin to grow the vesicular type becomes very prominent, although the granules are also found side by side (Text-fig. 11). Each vesicle shows

a thick jet-black rim and a lighter central area. Very rarely we



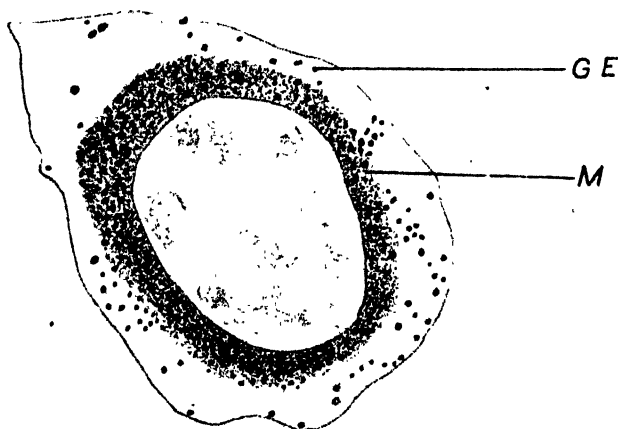
TEXT-FIG. 7.

Oogonium. Kolatchev and acid fuchsin. $\times 1200$.



TEXT-FIG. 8.

Kolatchev and acid fuchsin. $\times 1200$.

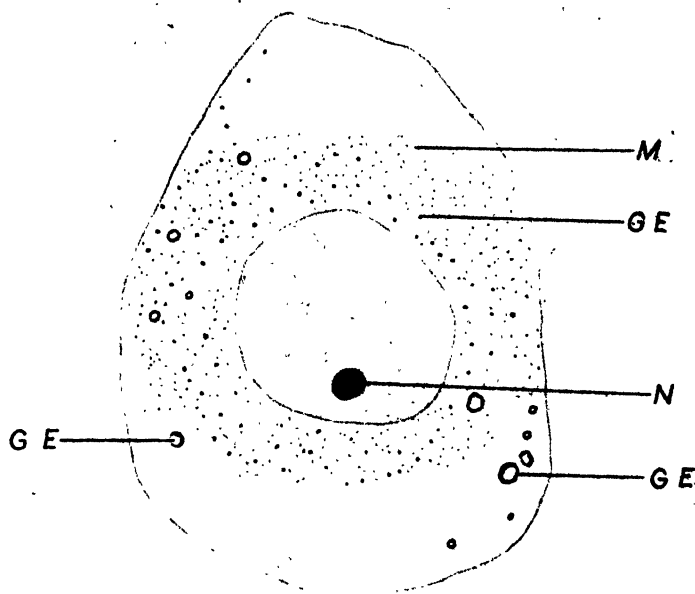


TEXT-FIG. 9.

Kolatchev unstained. $\times 1200$.

have come across in the Kolatchev preparations the crescentic type of Golgi element, each crescent showing an osmiophobe

area on its concave side (Text-fig. 10). We are forced to interpret these either as optical sections of vesicles or as those vesicles in which only a part of the rim is impregnated. From this it must not be concluded that the crescents are always the result of artifacts. The work of Gatenby, Bowen, and Hirschler, &c., has very clearly shown that the dyctyosomal or the crescentic type

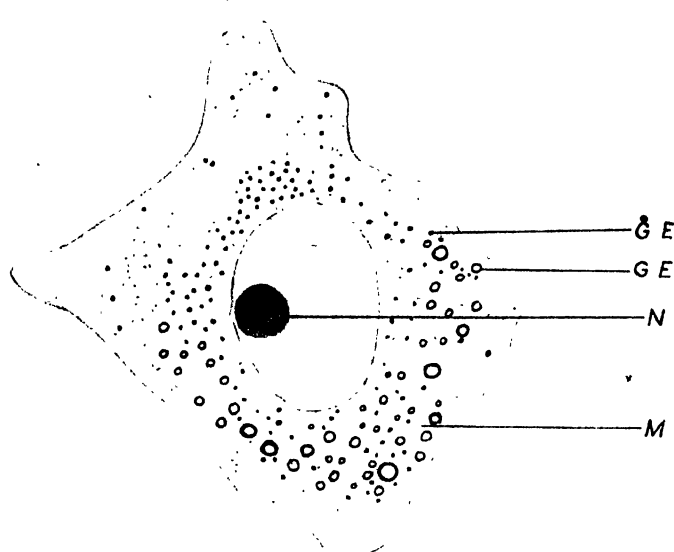


TEXT-FIG. 10.

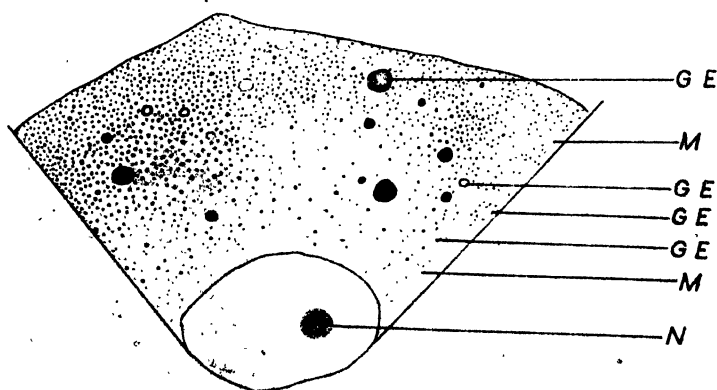
Kolatchev unstained. Golgi 'crescents' appearing at two places.
× 850.

of the Golgi element is a constant feature of the male germ-cells.

It has already been mentioned that in the course of oogenesis the Golgi elements not only grow in size but become more and more fatty. Consequently they appear as black bodies not only in Kolatchev but in Champy-fixed preparations also. For the same reason a large number of them are quickly decolorized

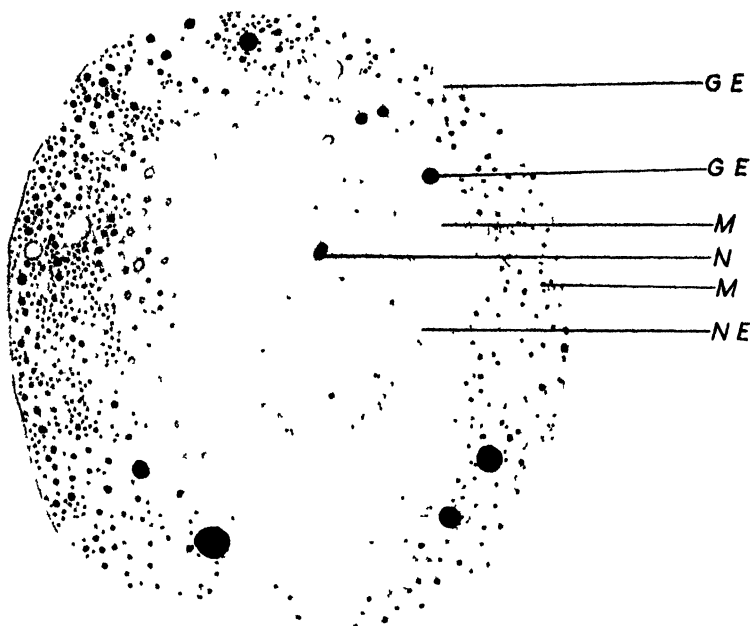


TEXT-FIG. 11.
Kolatchev unstained. $\times 520$.



TEXT-FIG. 12.
Kolatchev unstained. Some of the fatty Golgi elements
(fatty yolk) have been decolorized.. $\times 370$.

by turpentine or the xylol of Canada balsam and subsequently appear as empty spaces in the cytoplasm (Text-figs. 12 and 13). In Bouin's preparations also the Golgi vesicles appear as empty



TEXT-FIG. 13.

Champy stained with acid fuchsin. Many of the fatty Golgi elements (fatty yolk) have been decolorized. $\times 450$.

spaces as the result of their fatty contents having been washed out by acetic acid (Text-figs. 14-18).

(2) Nucleolus and Nucleolar Extrusions.

For the study of the nucleolus and nucleolar extrusions slides with Bouin and iron-haematoxylin technique were prepared. In a young oocyte (Text-fig. 14) there are a number of very darkly staining nucleoli lying in the nuclear meshwork. Similar

bodies staining sharply with haematoxylin occur on the nuclear membrane and in the cytoplasm. These have been interpreted as nucleolar material which has been extruded into the cytoplasm. Whether the nucleoli pierce the nuclear membrane as whole bodies or pass out in the form of a liquid into the cytoplasm where they are subsequently condensed it is difficult to tell. But the fact that we have seen the nucleolar extrusions impinging on the nuclear membrane even in the fresh cover-slip preparations (Text-figs. 2, 19, and 20) seems to suggest that they pass out as whole bodies.

During the earlier part of the growth period of the oocyte the



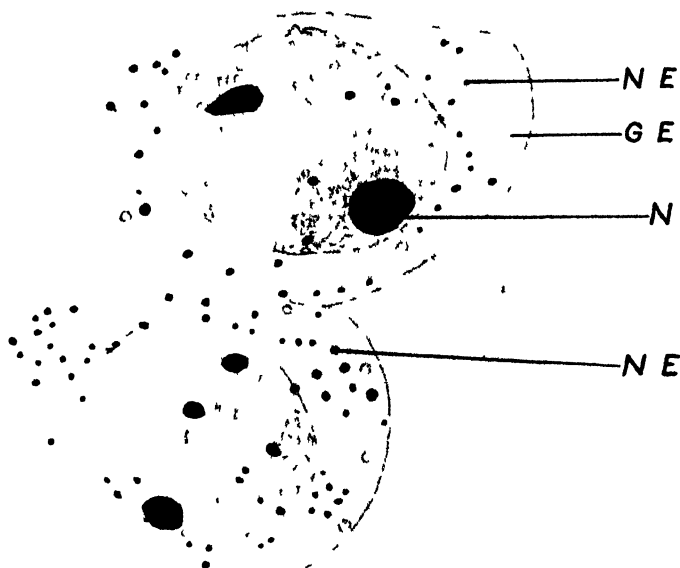
TEXT-FIG. 14.

Bouin and iron-haematoxylin. A negative picture of the Golgi elements appearing. $\times 1200$.

nucleolar extrusions are uniformly distributed in the cytoplasm (Text-figs. 15 and 16), but in later stages of oogenesis they are restricted to the perinuclear region (Text-figs. 13, 17, and 18). We thus recognize two series of nucleolar extrusions. Those of the first are much bigger. Appearances suggest that the extrusions of the first series are dissolved out in the cytoplasm. Hereafter very small extrusions only can be found. These are restricted to the perinuclear region and stain intensely with haematoxylin, like the small nucleoli themselves.

The nucleolar extrusions must not be confused with the mitochondria to be described later. Although the origin of albuminous yolk in the prawn is dealt with below, we find it necessary to state here that the nucleolar extrusions do not give rise directly to such yolk. We are driven to this conclusion because the extrusions remain restricted to the perinuclear region and the yolk appears for the first time in the peripheral

regions of the cytoplasm. This does not necessarily rule out the possibility of the extrusions indirectly contributing towards yolk by going into solution which subsequently reaches the periphery where yolk is elaborated.



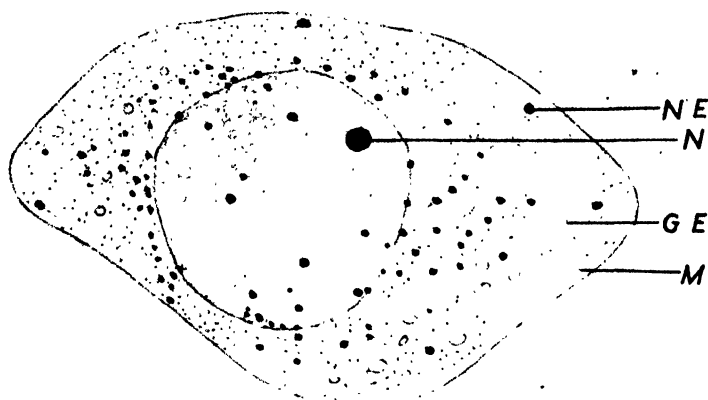
TEXT-FIG. 15.

Boun and iron-haematoxylin. A negative picture of the Golgi elements appearing. $\times 1200$.

(3) Mitochondria and Albuminous Yolk.

In the oonium we have been unable to discover any granules which we could assign to the category of mitochondria. At the inception of the growth period, however, the mitochondria appear either in the form of a heap lying on one side of the nucleus (Text-fig. 19) or in the form of a horseshoe closely embracing the nucleus (Text-fig. 2). In the mitochondrial mass small, closely aggregated granules of a white-greyish colour can be observed even in the absolutely fresh cover-slip preparations. In osmicated cover-slip preparations they appear yellowish, and

janus green B tinges them only slightly. Soon the juxta-nuclear mitochondrial mass becomes circum-nuclear (Text-fig. 9). Gradually the mitochondrial ring expands towards the periphery of the oocyte till the outer margin of the ring touches the limiting membrane of the egg (Text-figs. 20, 22, and 23). At the same time the peripheral mitochondria begin to swell up (Text-figs. 20 and 22), although rarely the more internal mitochondria may likewise grow in size (Text-fig. 23). We desire to



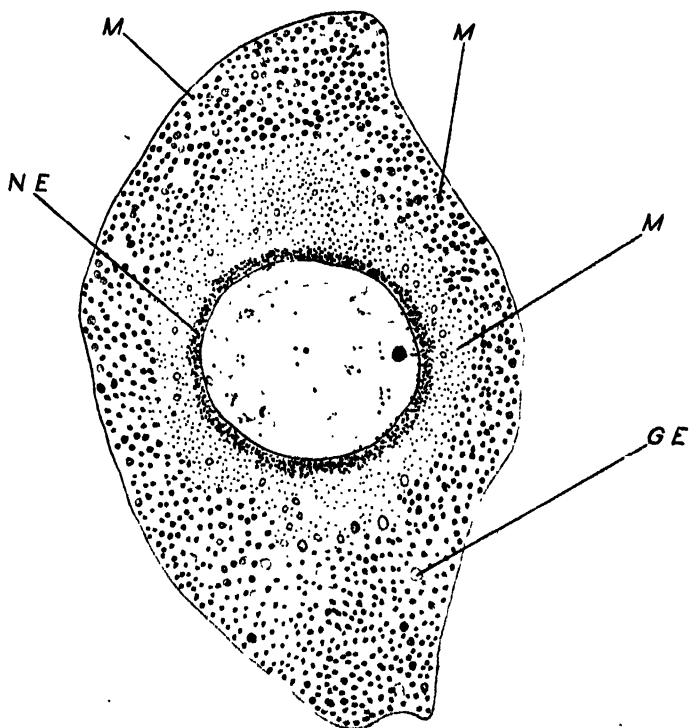
TEXT-FIG. 16.

Bouin and iron-haematoxylin. $\times 1200$.

lay stress on this highly characteristic feature of the mitochondria of the prawn. We have repeatedly seen the peripherally expanding mitochondrial ring with the swollen granules at its margin in fresh cover-slip preparations. This observation is principally responsible for our view that in the prawn albuminous yolk arises from the mitochondria.

Our belief that the swollen granules at the periphery of the mitochondrial ring are in fact mitochondrial in origin is further strengthened by the fact that in Bouin's preparations they appear as very much corroded granules and stain very faintly with haematoxylin (Text-fig. 17). This reaction is in accord with the general belief that mitochondria are made of lipo-proteins.

On the other hand, in Champy preparations stained with acid fuchsin (Text-fig. 13) they are fixed and stained excellently, appearing as perfectly round bodies. In Kolatchev unstained



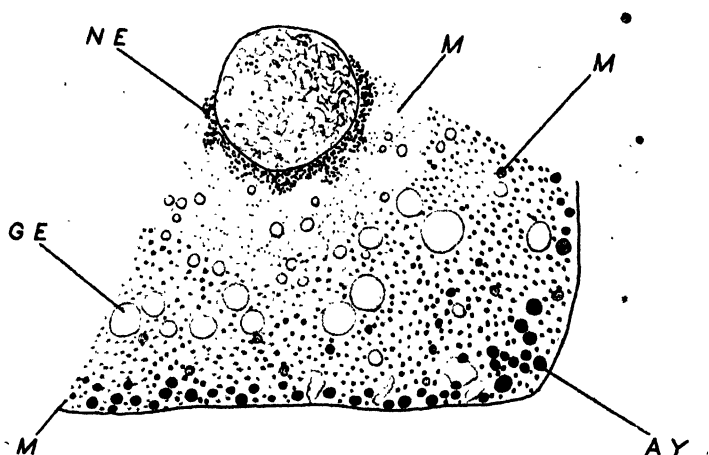
TEXT-FIG. 17.

Bouin and iron-haematoxylin. Yolk-forming mitochondria showing internal differentiation. $\times 420$.

preparations also they are fixed well and appear yellowish (Text-fig. 12).

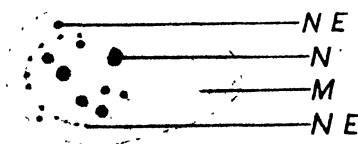
Amongst the swollen mitochondria now appear certain bodies which show an internal differentiation (Text-figs. 17 and 18). Some of these bodies seem to contain a number of small granules whereas others seem to show tiny vacuoles inside them. In Bouin-haematoxylin preparations these bodies are but slightly

better fixed and stained than the swollen mitochondria. Acid fuchsin following Champy fixation does not properly show this



TEXT-FIG. 18.

A portion of an oocyte measuring 0.3 mm. Albuminous yolk appearing at the periphery. A negative picture of the fatty yolk appearing. $\times 270$. Bouin and iron-haematoxylin.

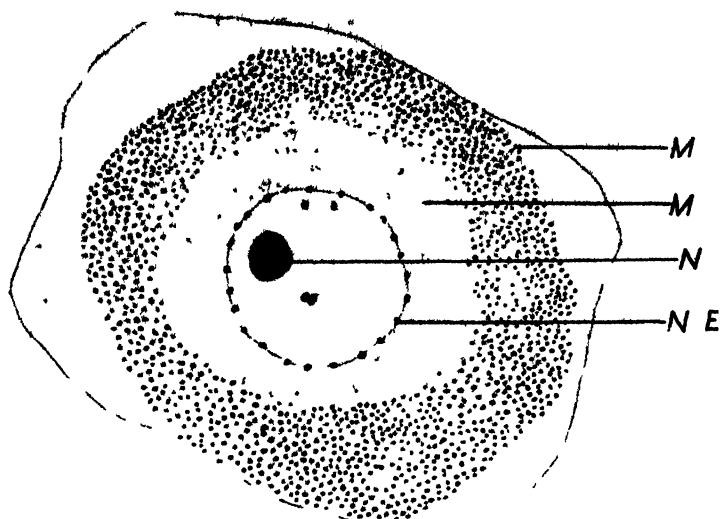


TEXT-FIG. 19.

2 per cent. osmic acid for three hours. $\times 355$.

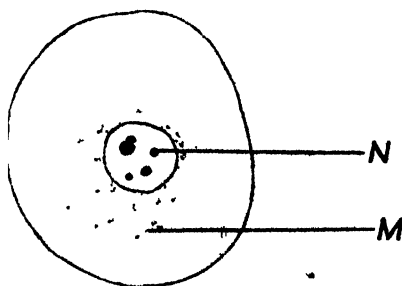
internal differentiation, although it can be seen in fresh cover-slip preparations (Text-fig. 4).

What is the origin of these bodies showing internal differentiation? Do they arise *de novo* in the cytoplasm or do they represent some of the swollen mitochondria which are being gradually shorn of their lipoidal constituents and are condensing albuminous material inside them? Certain it is that



TEXT-FIG. 20.

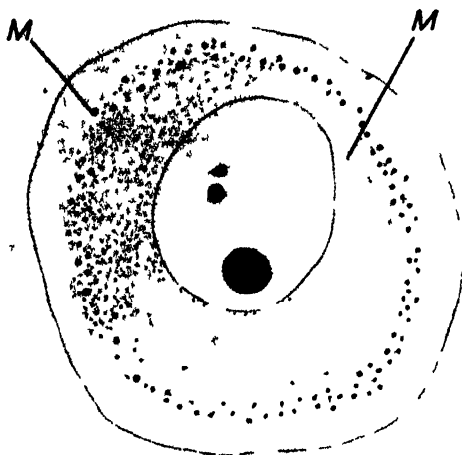
2 per cent osmic acid for $3\frac{1}{2}$ hours. $\times 355$.



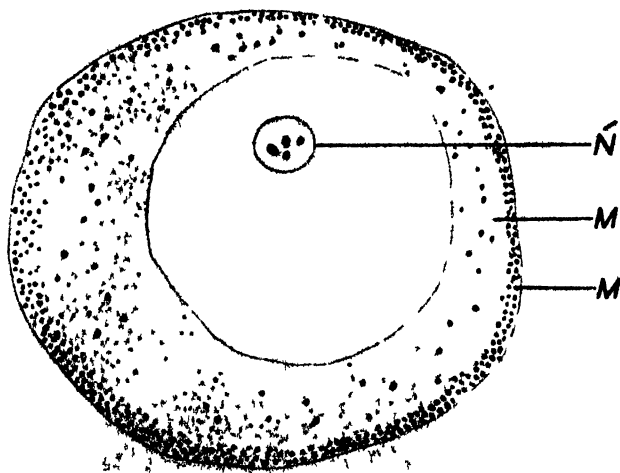
TEXT-FIG. 21.

A young oocyte of a prawn kept in Janus Green B for 20 hours. $\times 355$.

they are the precursors of albuminous yolk. Gradually they grow in size, lose their internal differentiation, and are now

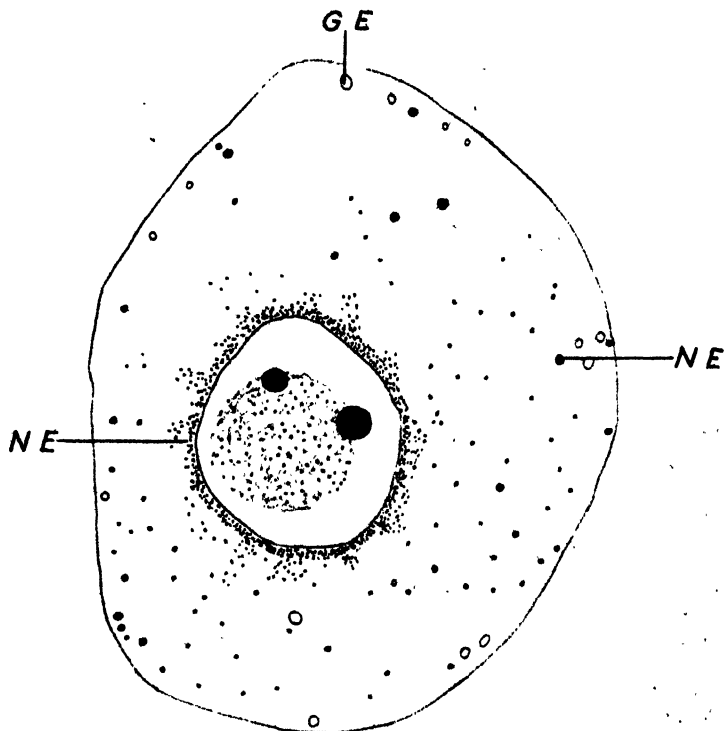


TEXT FIG 22
Absolutely fresh oocyte $\times 355$



TEXT-FIG 23
An oocyte of a prawn kept in Janus Green B for 20 hours.
 $\times 355$.

excellently fixed in Bouin and are stained deeply with haematoxylin. They now move towards the extreme periphery of the oocyte and represent the albuminous yolk *sensu stricto*



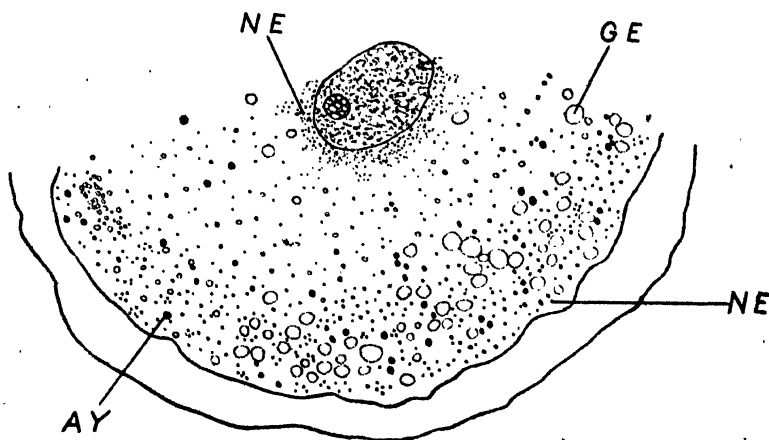
TEXT-FIG. 24.

Bouin and iron-haematoxylin. A negative picture of the fatty Golgi elements appearing. $\times 725$.

(Text-fig. 18). The yolk invariably makes its appearance in the peripheral regions of the cytoplasm, but during the later stages of oogenesis it is deposited more and more internally till all the regions of the cytoplasm are packed with it. At the same time the yolk bodies grow in size (Text-fig. 5).

We are inclined to interpret the bodies showing internal

differentiation as the transforming mitochondria for the following reasons. Firstly, they appear amongst the swollen mitochondria. Secondly, they are fixed and stained poorly in Bouin iron-haematoxylin like the swollen mitochondria. Thirdly, experience shows that whenever albuminous yolk arises *de novo* in the cytoplasm, e.g. in *Crossopriza* and *Culex* (Nath, 1928 and 1929), it arises in the form of very small



TEXT-FIG. 25.

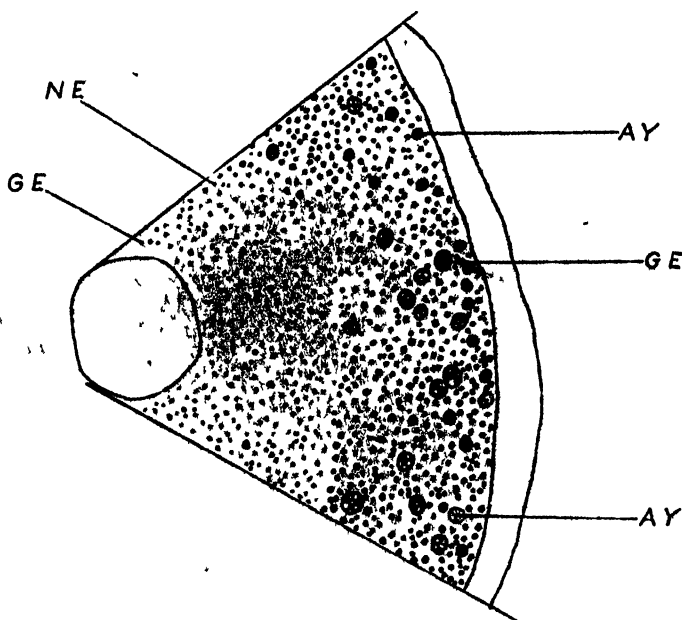
A portion of an oocyte measuring about 0.3 mm. Bouin and iron-haematoxylin. $\times 270$. Nucleolar extrusions wandering towards the periphery to form yolk.

granules which are fixed excellently in fixatives containing acetic acid and stain brilliantly with iron-haematoxylin.

Paratalphusa spinigera.

In spite of our best efforts we have failed to obtain very young oocytes of the crab. Our account begins from an oocyte that measures about 0.1 mm. We are therefore unable to describe the distribution of the Golgi elements and the mitochondria in the earliest stages of oogenesis. This, however, has not prevented us from giving a full account of the origin of yolk, which is usually deposited at an advanced stage in oogenesis.

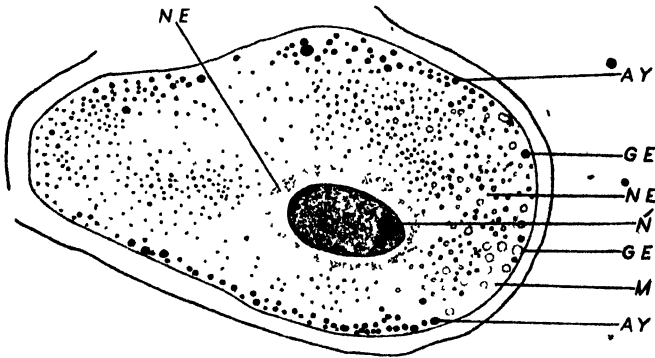
In the prawn it will be remembered that the nucleolar extrusions of the first series are large and are uniformly distributed in the cytoplasm. These soon disappear and hereafter very much smaller extrusions of the second series can be constantly observed arranged in a circum-nuclear fashion. These latter



TEXT-FIG. 26.

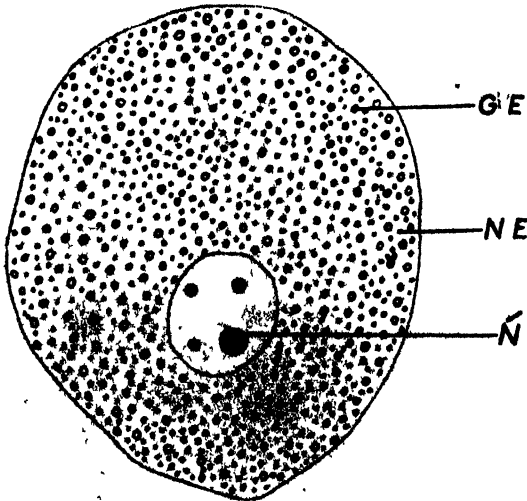
A portion of an oocyte measuring about 0.5 mm. Champy and acid fuchsin. Fatty Golgi elements black. Nucleolar extrusions forming yolk at the periphery. $\times 270$.

remain restricted to this area and have never been seen wandering into the cytoplasm. In the youngest available oocyte of the crab also (Text-fig. 24; Bouin and haematoxylin) there exists a circum-nuclear ring of sharply staining granules which are exactly similar to the granules suspended in the nuclear mesh-work which is here contracted. These have been interpreted as the nucleolar extrusions. This circum-nuclear ring continues



TEXT-FIG. 27.

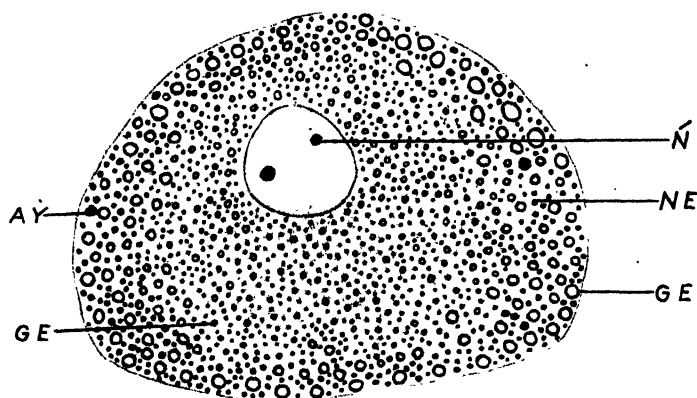
Champy and acid fuchsin. Some of the fatty Golgi elements appear black, others have been decolorized and appear as hollow circles. Some of the nucleolar extrusions forming yolk at the periphery. $\times 360$.



TEXT-FIG. 28.

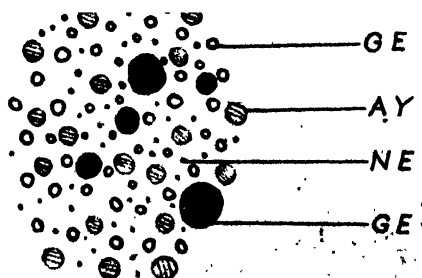
2 per cent. osmic acid for 4 hours. $\times 468$.

to exist in older oocytes (Text-figs. 25 and 27) as in the prawn. Unlike the prawn oocyte, however, granules from this ring wander away into the cytoplasm, where they begin to grow and



TEXT-FIG. 29.

2 per cent. osmic acid for 4 hours. Nucleolar extrusions forming yolk at the periphery. $\times 270$.

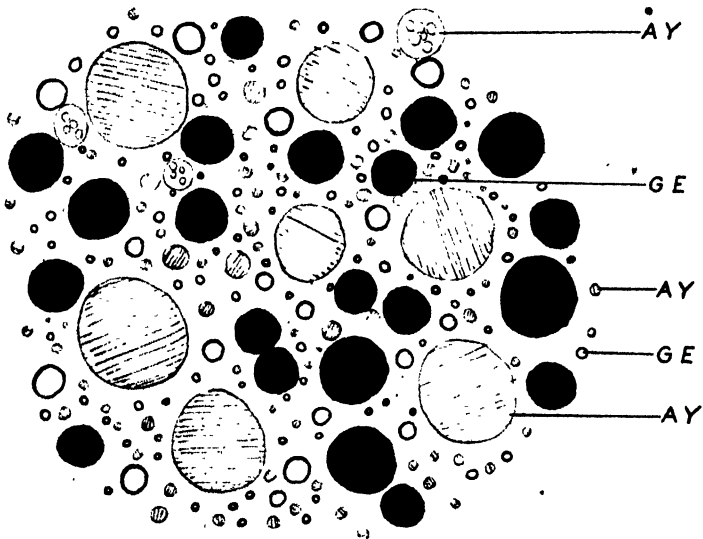


TEXT-FIG. 30.

Contents of an oocyte measuring 1.3 mm. kept in 2 per cent. osmic acid for 25 minutes. $\times 200$.

directly give rise to albuminous yolk which appears for the first time, as in the prawn, in the peripheral regions of the cytoplasm (Text-fig. 25; Bouin and haematoxylin). In this figure may be studied all gradations between a small, sharply staining

nucleolar extrusion and the definitive yolk. During growth some, if not all, of the yolk spheres show a vacuolated appearance (Text-figs. 26 and 31). A similar phenomenon has been recently described by Gresson (1929) in the saw-flies and was observed by the senior author in *Luciola*, although it was not



TEXT-FIG. 31.

Contents of an oocyte measuring 3 mm. kept in 2 per cent. osmic acid for 10 minutes. $\times 150$.

recorded. The mitochondria which seem to play such an important rôle in the formation of the albuminous yolk of the prawn oocyte are here remarkably inactive. They can be here demonstrated as very small fuchsinophil granules evenly distributed in the cytoplasm (Text-fig. 27).

The Golgi elements of the crab oocyte, on the other hand, are exactly similar to those of the prawn oocyte, and they can be likewise studied in an eminently satisfactory manner in coverslip preparations osmicated for brief periods (Text-figs. 28 and 29). Each element shows a dark rim and a slightly brownish

central area. As in the prawn oocyte, many of the Golgi vesicles of the crab grow enormously in size and become more fatty in the course of oogenesis. Consequently they are blackened in shorter periods of osmication (Text-figs. 30 and 31).

DISCUSSION.

In spite of what has been said by Harvey (1929) to the contrary, one thing that has emerged most clearly from the investigations carried out in this laboratory during the last few years is that the Golgi apparatus generally gives rise in oogenesis to the fatty yolk, which is distinct, both morphologically and chemically, from the ordinary albuminous type of yolk. Apart from the papers which have been cited by Nath (1930) in support of this claim, we draw particular attention to the recent work of Gresson (1929) and Bell on the oogenesis of the saw-flies and the dog spermatid respectively. Gresson has confirmed in every detail Nath's account of the origin of fatty yolk from the Golgi elements. The detailed evidence in support of this claim has been set forth by Nath (1930 and 1931) and we refrain from repeating the same. Here we desire merely to mention that both in the prawn and the crab the Golgi elements and the fatty yolk are morphologically similar inasmuch as both are vesicular. The reaction of the Golgi elements to osmic acid indicates that they are lipoidal. In the course of oogenesis *pari passu* with their growth they gradually develop neutral fats inside them, with the result that they are blackened by osmic acid in shorter and shorter periods. All gradations exist between the tiny Golgi vesicle and the enlarged fatty yolk sphere (see Observations).

The recent work of Bell has shown that even in the male germ-cells neutral fats are developed from the lipoidal Golgi elements. In the young spermatid of the dog the Golgi elements exist in the form of osmiophilic granules which for some period increase in number, but later on some of them aggregate to form large spheres of neutral fat. Space does not permit us to cite the most convincing evidence based on well-established facts of chemistry which Bell has advanced in favour of the view that phospho-lipins, of which the Golgi material is probably

made, are transformed into neutral fat. For details reference may be made to the original paper (p. 619).

We now turn to the consideration of the claims that in *Perca* and *Pygosteus* (Hibbard and Parat, 1927 and 1928), in *Discoglossus* (Hibbard, 1928), in *Lygia* (Steopoe, 1929), and lastly in *Carcinus* (Harvey, 1929), the Golgi apparatus gives rise to the albuminous yolk and that fat arises *de novo* in the cytoplasm. About the first three claims we will say very little. The introductory pages of this paper have made it sufficiently clear that Parat's, Hibbard's, and Steopoe's Golgi apparatus (the vacuome) is not the true lipoidal Golgi apparatus but consists admittedly of a number of aqueous, 'acidic', non-lipoidal, and neutral red-staining discrete vacuoles. The recent work of Nath (1930 and 1931) and of Nath and Nangia (1931) on the frog and the teleostean fishes respectively has confirmed the origin of albuminous yolk from the vacuome, and they have also demonstrated the true lipoidal Golgi apparatus lying side by side with the vacuoles and mitochondria in the absolutely fresh material.

The 'lepidosomes' or the modified mitochondria of Parat have been conclusively shown to be the true lipoidal Golgi apparatus in male germ-cells by Gatenby, Hirschler, Voinov, &c.; and we strongly suggest that the 'lepidosomes' described by Steopoe in *Lygia* probably represent the same substance.

A very careful study of Harvey's paper, however, has convinced us that he is dealing with a true lipoidal Golgi apparatus and not with the vacuome. In *Carcinus*, according to Harvey, the Golgi elements can be seen with considerable difficulty in unstained living material as very thin, slightly refractive rodlets, usually with a slight curve, but some have been observed to be almost of horseshoe shape. These rodlets probably represent the chromophil rim, or part thereof. It is obvious that Harvey is not dealing with Parat's vacuome, because an aqueous vacuole cannot possibly appear as a rodlet in the living material. He is evidently dealing with the classical lipoidal Golgi apparatus. Now according to Harvey albuminous yolk arises in relation with the Golgi bodies, and probably is

deposited in the chromophobe part thereof under the influence of the Golgi apparatus and probably of the mitochondria. Later the material of the nucleolar extrusions is also added to the yolk droplets. We readily accept the suggested role of the mitochondria and the nucleolar material in *Carcinus*, but we are unable to accept the view that the Golgi material, which is admitted by all except the Parat School to be lipoidal in constitution, gives rise to such a different material as the albuminous yolk. On the other hand, apart from the morphological evidence advanced by Nath, lipoids and fats are in some respects closely related substances, and the theory of the origin of the one from the other does become readily acceptable.

Oniscus (King, 1926) and the prawn described here closely resemble each other as to the Golgi origin of fatty yolk and the mitochondrial origin of proteid yolk. In both cases there is at one time a peripheral zoning of mitochondria swelling into yolk (cf. *Rana tigrina*, Nath, 1931); but in *Oniscus* the nucleolus does not throw out any material which can be discerned with the aid of the microscope.

In the prawn, on the other hand, there is a well-marked nucleolar activity. But after the disappearance of the nucleolar extrusions of the first series, those of the second remain restricted to the perinuclear region and are not to be found in the peripheral regions of the cytoplasm where the mitochondria are gradually transforming into albuminous yolk. A direct origin of a yolk granule from the nucleolar extrusion, as has been described, for example, in *Saccocirrus* (Gatenby, 1922), in the cockroach (Hogben, 1920; and Nath and Piare Mohan, 1929), in *Luciola* (Nath and Mehta, 1929), and in *Dydercus* (Bhandari and Nath, 1930), must therefore be ruled out in the case of the prawn. A perinuclear zone of nucleolar extrusions is also established in *Paratalphusa*, but the particles continue to wander away into the cytoplasm at the periphery of which they directly grow into yolk, the mitochondria playing no visible part in its formation.

Lastly, we suggest that the vacuolated appearance of the yolk-forming mitochondria in the prawn and of the yolk-forming nucleolar extrusions of the crab, the saw-flies, *Luciola*,

and the cockroach are but manifestations of the process of growth which these granules have to undergo to form yolk.

SUMMARY.

Palaemon lamarrei.

1. In the oogonia there are no granules which can be assigned to the category of mitochondria. They appear for the first time in young oocytes in the form of a juxta-nuclear heap of granules or in the form of a horseshoe closely embracing the nuclear membrane. Soon they arrange themselves in the form of a circum-nuclear ring which gradually expands towards the periphery of the oocyte without breaking away from the nuclear membrane. At the same time the marginal mitochondria of the ring grow in size till ultimately they give rise to albuminous yolk, which therefore appears for the first time in the peripheral regions of the cytoplasm (cf. *Oniscus*, King, 1926, and *Rana tigrina*, Nath, 1931).

2. A yolk-forming mitochondrium first swells up; but it is still poorly fixed and stained with Bouin-haematoxylin, like the unchanged mitochondria. The process of growth continues and the swelling mitochondria now show an internal differentiation in the form of minute granules or very small vacuoles. Such mitochondria are only slightly better fixed and stained with Bouin-haematoxylin. Gradually they are completely shorn of their lipoidal constituents, condensing at the same time more and more of protein material. Ultimately they give rise to albuminous yolk, *sensu stricto*, which is fixed and stained excellently in Bouin-haematoxylin.

3. In the earliest oocytes the nucleolus throws out into the cytoplasm deeply basophil pieces which are more or less uniformly dispersed. Soon they disappear. Hereafter the nucleolar extrusions are very minute, but they remain restricted to the perinuclear region. They never wander into the general cytoplasm or at least into its peripheral regions where protein yolk appears for the first time. A direct origin of the yolk granule from the extrusion must, therefore, be ruled out. But the

possibility of the extrusions going into solution and thus indirectly contributing towards yolk cannot be eliminated.

4. Although the mitochondria can be easily observed in the fresh cover-slip preparations of young oocytes, the Golgi elements cannot be demonstrated unless the material is osmicated for at least twenty-two hours. Chemically the Golgi elements are lipoidal (fat-like). They are not stainable with neutral red.

5. In the oogonia and the earliest oocytes the Golgi elements exist in the form of vesicles, each vesicle showing a thick osmiophilic cortex and a central osmiophobic area.

6. During oogenesis many vesicles grow enormously in size, store up neutral fats inside them, and give rise to the fatty yolk as in *Lithobius*, spider, *Otostigmus*, *Luciola*, cockroach, *Dysdercus*, and *Ophiocephalus* (Nath, and Nath and collaborators), in *Oniscus* (King), in saw-flies (Gresson), and in *Helix* (Brambell).

7. The vacuolar system is absent in the prawn and also in the crab.

Paratالpusa spinigera.

8. The Golgi elements of the crab behave exactly like those of the prawn, but the mitochondria, on the other hand, remain inactive and have no visible relationship with yolk formation.

9. In the crab also there are well-marked nucleolar extrusions. As in the prawn a prominent circum-nuclear ring of these granules is established early in oogenesis. But, unlike the prawn, granules from this ring continue to wander into the cytoplasm at the periphery of which they directly grow into the albuminous yolk.

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On the Nature of the Trabecula Cranii.

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With Plates 40-6 and 3 Text-figures.

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1. INTRODUCTION TO THE PROBLEM.

THE trabeculae of the chondrocranium, discovered first by Rathke (1839) in the grass-snake, were recognized by W. K. Parker as the essential and fundamental pair of elements of the anterior region of the cartilaginous skull, just as the cartilages which Huxley (1874) termed parachordals were the basis from which the posterior region of the skull arose. But whereas the nature of the parachordal cartilages as parts of the axial skeleton, flanking the notochord, and developed from the sclerotomes of the mesodermal somites of the head, has never been in doubt, the nature of the trabeculae is still a matter for discussion.

Huxley (1874 and 1875) regarded the trabeculae as part of the splanchnocranium, and as belonging to the same class of structure as the visceral arches. Parker (1878) was first of the opinion that only the facial or anterior part of the trabeculae

was of visceral origin; then he accepted Huxley's view that the whole of the trabecular rods was visceral; and finally he returned to his first opinion. Platt (1898, p. 422) thought that 'the trabeculae were primarily bars of a prae-oral visceral arch', and, more recently, Allis (1923, 1924, and 1931), has upheld the view that the trabeculae are visceral. While he identifies the posterior portion or polar cartilage of van Wijhe (1904) as the pharyngeal element of the mandibular visceral arch, he considers that the anterior portion or trabecula *sensu stricto* represents the dorsal half of the premandibular arch.

Against these opinions, it must be mentioned that van Wijhe (1922) and Goodrich (1930) are disinclined to accept the view that the trabeculae are visceral, and consider them as structures *sui generis*, developed in relation to the forebrain.

The problem may therefore be stated in the following terms: (1) Are the trabeculae cranii portions of the chordal skeleton, formed from the sclerotomes of mesodermal somites, and of a nature similar to that of the parachordal cartilages? And if not: (2) Are the trabeculae cranii visceral structures, formed from the mesenchyme of the visceral arches, and of a nature similar to that of the splanchnocranium?

The answer to the first of these questions necessitates an excursion into a number of fields of zoological inquiry, and foremost among these is the problem of the localization of the morphological anterior extremity of the head, and its relation to the foremost point of the axial skeleton.

It has been held for some time that the true morphological anterior extremity of the head of a chordate animal is to be found in the vicinity of that region in which the hypophysis, the hinder point of closure of the neuropore, the pre-oral gut (Seessel's pocket), the tip of the notochord, and the transverse commissure between the two premandibular somites are to be found. This region is, therefore, to be regarded as derived from the animal pole of the egg: a view which is supported by experimental results. The observations of Stockard (1910) on the effects of certain toxic substances to which very early stages of development of the fish *Fundulus* are subjected show that fish so treated are deficient in that they lack the optic chiasma

and the region between the eyes, and present the appearance of cyclopia. It is to be noted that the missing region corresponds very closely to that which is held on morphological grounds to represent the anterior end of the head. These results have been confirmed in the frog by Bellamy (1919). Treatment with toxic substances is known to exert a differential effect on the tissues of the animal pole of the egg and, since this treatment has resulted in the absence of certain structures, there is experimental evidence for the view that the missing region (optic chiasma and its neighbourhood) represents the morphological anterior extremity of the head.

Attention may now be turned to the question as to how far forward the axial skeleton extends. Since it is derived from the sclerotomes of the mesodermal somites, its anterior end must be looked for in the region of the first somite, which is situated in the first or premandibular segment. It is to be remembered that there is firm evidence for homologizing the premandibular somites of Craniates with the anterior head-cavities of *Amphioxus* (Goodrich, 1917), and therefore there is no ground for the view that there ever were any segments in front of that which is recognized in Craniates as the premandibular segment.

The premandibular somites are interconnected with one another across the middle line by a commissure which is formed by being pulled off from the roof of the pre-oral gut by the tip of the notochord (de Beer, 1926). This commissure is therefore situated precisely in the region regarded as the anterior end of the head, and it has been observed to coincide closely with the structure known as the acrochordal, and even to give rise to it. The acrochordal does not chondrify in *Selachii* (van Wijhe, 1922), but it does in birds (Sonies, 1907, and Jager, 1924). Now the acrochordal forms the *dorsum sellae turcicae* or ridge overhanging the pituitary fossa from behind, and it is situated at the extreme front end of the basal or parachordal plate. Further, the acrochordal is situated in line with the *pilae antoticae* and is continuous with the base of these structures, which, themselves, are attached to the foremost edge of the basal plate on each side. There is, therefore, definite evidence that the most anterior part of the chordal skeleton is formed by the acrochor-

dal, at the front of the parachordal or basal plate. It follows, therefore, that since the trabeculae are in front of the acrochordal, whatever they may be, on the existing evidence they are probably not part of the chordal or sclerotomic skeleton.

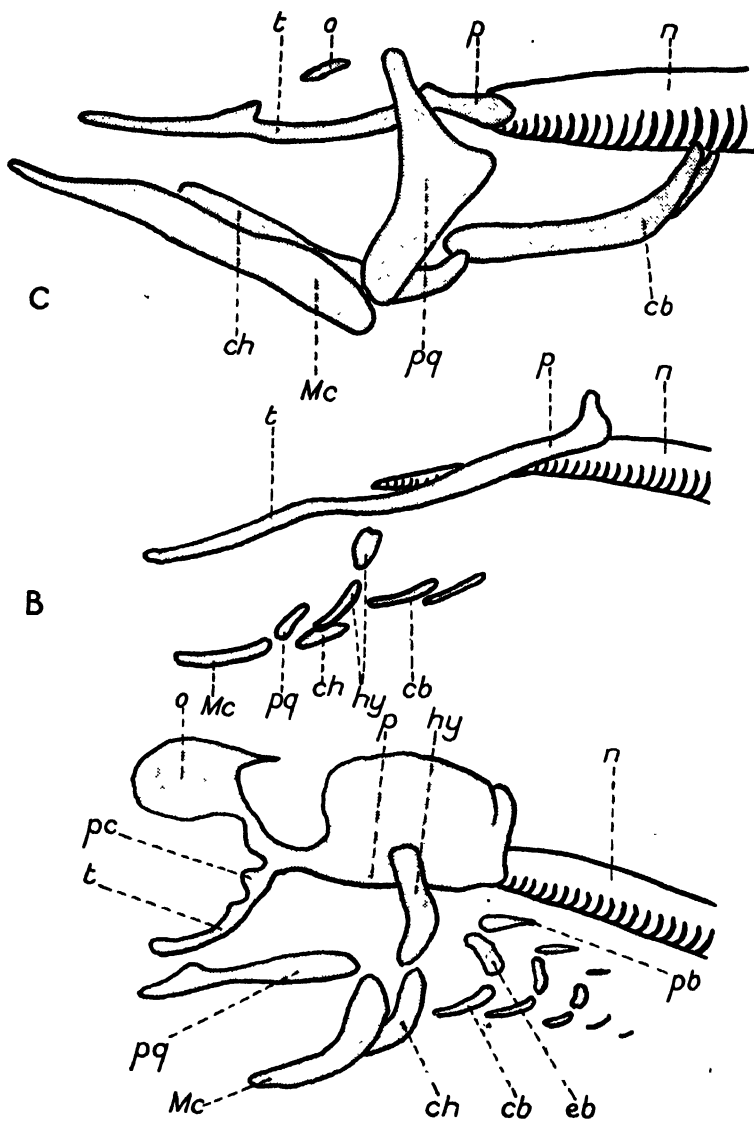
Attention may now be turned to the second question formulated above. If the trabeculae are of visceral origin, they must form part of a visceral arch in front of the mandibular arch. That such a premandibular arch did exist in early Chordates would seem to follow from Stensiø's descriptions of certain Ostracoderma. Now, if a pair of premandibular arches were present in the embryo of a Craniate, it is difficult to see what region they would occupy other than that which is actually taken up by the trabeculae. It might be said that the suitability of the position of the trabeculae in Selachian embryos for regarding them as visceral arches is assisted by the pronounced cranial flexure, as a result of which the trabeculae lie almost at right angles to the parachordals and parallel with the recognized visceral arches. But in the Teleostei, Anura, and Urodela, where the trabeculae arise almost in line with the parachordals, their position is no less suitable for regarding them as a pair of premandibular visceral arches, and, curiously enough, in these forms the recognized visceral arches slope forwards and downwards in such a way that they are not far off parallel with the trabeculae. These relations are indicated in Text-fig. 1.

Allis's (1923, 1924, and 1931) claim that the trabeculae are visceral is based on morphological considerations. Arguing from the fact that the position of the primitive wall of the skull as a brain-case is indicated by the thick meningeal membrane or dura mater, he has shown that a space—the subpituitary space—through which the pituitary vein passes, is external and ventral to the dura mater, although it is enclosed above the trabeculae in that cavity, which, neglecting the dura mater, is usually and loosely known in the dry skull as the cranial cavity. The trabeculae are attached to the primitive wall of the skull—the parachordals—behind. In front of this point the trabeculae enclose the subpituitary space between themselves and the dura mater. In front of this, again, the trabeculae bend upwards and come into contact with the dura mater. These relations (which

are indicated in the text-figures illustrating the writer's paper on the Development of the skull of *Scyllium*) are impossible to understand if the trabeculae are regarded as elements forming part of the original cranial wall, whereas they become easy to interpret if the trabeculae are visceral arches which have become attached by their dorsal and posterior end to the front of the chordal skeleton—the parachordals—and have entered into relations with the forebrain.

A large part of the forebrain, including all structures in front of the recessus preopticus, must be regarded as the result of a forward expansion of dorsal regions of the neural tube which originally lay behind the morphological anterior extremity of the head. In forms in which the forebrain is but slightly developed, the cartilages of the chordal skeleton—the parachordals—would be sufficient to form a foundation for the neurocranium. As will be shown later, there is reason to believe that the *Cyclostomata* are in this primitive condition. But with the expansion and enlargement of the forebrain which is characteristic of *Gnathostomata*, the concurrence of the trabeculae would become necessary for the enclosure of the brain. It may be urged as an objection to this view that it is difficult to understand how or why a visceral structure could enter so intimately into the formation of the brain-case as does the trabecula. To this may be answered, first, that an analogous case is provided by the processus ascendens, ala temporalis, and Mammalian alisphenoid, structures of undisputed visceral origin which have nevertheless come to play an important part in the limitation of the brain-case; and, second, that the trabeculae invariably arise as elements separate and distinct from the parachordals: a fact which is so commonplace as to be overlooked, and which indicates that the intimacy of the relations into which the trabeculae eventually get with the parachordals must not be exaggerated.

Thus far Allis's case is strong and his theory falls into line with a number of other views. But he goes farther, and wishes to identify the polar cartilage with the pharyngomandibular, the trabecula *sensu stricto* with the dorsal half of the premandibular arch, and the palatine process of the pterygo-



TEXT-FIG. 1.

Diagram prepared from camera lucida drawings of victoria blue preparations of the chondrocranium showing the relation of the trabeculae to other structures in *A*, *Scyllium*; *B*, *Salmo*; and *C*, *Triton*.

EXPLANATION OF LETTERING.

as, auditory sac; *c*, remnant of transverse commissure between pre-mandibular somites; *ca*, cerebral artery; *cb*, ceratobranchial cartilage; *ch*, ceratohyal cartilage; *cs*, connecting strand between trabecula and mesenchymatous plaque; *e*, eye; *eb*, epibranchial cartilage; *er*, external rectus muscle; *fb*, forebrain; *fg*, foregut; *fn*, facial nerve; *gn*, glossopharyngeal nerve; *gs1-5*, gill-slit 1-5; *h*, hypophysis; *ha*, hyoid arch; *hb*, hind-brain; *hs*, hypophysial stalk; *hy*, hyomandibular cartilage; *i*, infundibulum; *io*, inferior oblique muscle; *ir*, inferior rectus muscle; *itr*, internal rectus muscle; *lj*, lower jaw; *m*, mesenchyme proliferated beneath hypophysis and forebrain by maxillary process; *ma*, mandibular arch; *mb*, midbrain; *Mc*, Meckel's cartilage; *mm*, visceral muscle-plate of mandibular arch; *mp*, maxillary process; *ms*, mandibular visceral cleft; *mv*, mandibular blood-vessel; *n*, notochord; *ns*, nasal sac; *nt*, neural tube; *o*, orbital cartilage; *on*, optic nerve; *p*, parachordal cartilage; *pb*, pharyngobranchial cartilage; *pc*, polar cartilage; *ph*, pharynx; *pl*, mesenchymatous plaque; *pma*, premandibular arch; *pn*, profundus nerve; *ppg*, peripharyngeal groove; *pq*, pterygo-quadrate cartilage; *rh*, remnant of hyoid pouch; *rMc*, rudiment of Meckel's cartilage; *roc*, rudiment of orbital cartilage; *rpc*, rudiment of polar cartilage; *rpq*, rudiment of pterygoquadrate cartilage; *rr*, rudiment of rostral cartilage; *rt*, rudiment of trabecula cranii; *s1*, first or premandibular somite; *s2*, second or mandibular somite; *sh*, side-wall of hypophysial sac; *sp*, spiracular slit; *spp*, spiracular pouch; *sr*, supra-rostral cartilage; *st*, stomodaeum; *stp*, lateral pouches of stomodaeum; *su*, ventral sucker; *t*, trabecula cranii; *tc*, transverse commissure between premandibular somites; *tn*, trigeminal nerve; *ul*, upper lip; *v*, velum; *vn*, vagus nerve.

quadrate cartilage with the ventral half of this arch. Here he is on much more uncertain ground; for one thing, there is another element which is a claimant for the position of pharyngomandibular (see Sewertzoff and Disler, 1924), and, for another, there seems to be no reason for regarding the palatine process of the pterygo-quadrate as anything but a secondary development of that cartilage. It seems wiser, therefore, not to burden the hypothesis of the visceral nature of the trabeculae with these speculations of more doubtful value.

Stone (1926) has performed experiments which, although difficult to interpret in one sense, nevertheless have an important bearing on the present problem. He removed the neural crest from the head of embryos of *Amblystoma*, and found that not only did such embryos show a deficiency in the cartilages of the visceral arches on the operated side, but that the

trabeculae were also deficient, and did not extend farther forward than the optic nerve. From the present point of view, it is unnecessary to consider the controversial question as to whether visceral cartilage is derived from the cells of the neural crest; the essential part of Stone's results is the fact that the operation has produced the same effect on the trabeculae as on the visceral arches, and no effect on undoubted sclerotomic or chordal cartilage. It may be inferred, therefore, that the trabeculae are of a nature different from that of the chordal cartilages and similar to that of the visceral cartilages.

From the considerations dealt with above, it will be apparent that there is a considerable body of inference on which to found a *prima facie* case for the view that the trabeculae are visceral structures. Attention may now be turned to the actual observations which have been made on the origin of the trabeculae. Such evidence is astonishingly meagre. Starting with the fish, Sewertzoff (1916, p. 17) states that in *Acipenser* the trabeculae do not arise from the premandibular somites, and (1917) that the sclerotomes of the foremost mesodermal somites in *Acipenser* give rise to the parachordals.

According to Haller (1923) the mandibular arch of the Selachian embryo (*Squalus*) is to be regarded as composed of four portions which are externally distinguishable. These are the Kieferaugenspaltstück, Oberkieferstück, Zwischenstück, and Unterkieferstück. The latter three portions contain material which will give rise to the skeleton of the mandibular arch (pterygo-quadrate and Meckel's cartilage). But the first or most dorsal portion is said to contain procartilage which gives rise to the posterior region of the trabecular bar, i.e. to the polar cartilage.

A pair of maxillary processes then make their appearance, growing inwards and forwards from the top of the mandibular arch, pushing their way between the skin and the floor of the brain. In this manner the floor of the brain comes to lie at some distance beneath the superficial epidermis, and Rathke's pocket or the hypophysial invagination becomes relatively deepened, as Woerdeman (1914), Haller, and Allis (1923) stated. The anterior portion of the trabecular bars (*trabecula sensu*

stricto) arises from mesenchyme in these maxillary processes, and must therefore be held to be of visceral origin.

That the origin of the trabecula in the Teleost is curious emerges from Lundborg's (1899) attempt to show that in *Salmo* it arose from the ectodermal lining of the roof of the stomodaeum! Further, he attributed the same method of origin to the trabecula in *Rana*. Concerning the latter animal, there is a careful study by Spemann (1898) which is, however, not concerned with the details of the origin of the mesenchymal condensations under consideration in the present study.

As regards *Urodela*, Sewertzoff (1916) states that his researches on these forms (as also Filatoff's on *Reptilia*) led him to the same conclusion as those on *Acipenser*, viz. that the trabeculae do not arise from the premandibular somites. Platt (1893, 1898) has devoted a considerable amount of study to the problem of the origin of the trabeculae and visceral skeleton of *Necturus*. She believes that the anterior part of the trabeculae and the cartilages of the visceral arches arise from mesenchyme, and not from segmental sclerotomes. It is true that she is also concerned with the origin of this mesenchyme from the neural crest, which is another question, with which the present paper has nothing to do. But, from the present point of view, the essential feature of Platt's results is that according to her the trabeculae (or rather the anterior part of them) arise from mesenchyme, and her results are confirmed in a remarkable way by Stone's (1926) experiments. Platt also draws attention to the fact that a histological distinction exists between the rudiments formed from the sclerotomes, which contain yolk-granules, and those which form the anterior part of the trabeculae and visceral arches, which contain no yolk-granules. Further, the nuclei in the cartilage derived from sclerotomes are farther apart than in the other. A similar histological distinction between the anterior part of the trabeculae and the cartilage of the basal plate was reported by Stöhr in *Triton* (1880). The structures which Platt calls 'the posterior part of the trabecular bars', and Stöhr the 'Balkenplatte', would seem to be nothing but the anterior horns of the parachordals.

In view of the difference of opinion and uncertainty which exists in regard to the nature of the trabeculae, it seemed advisable to reinvestigate the matter, and I have accordingly studied their origin in four types, viz. *Scyllium canicula*, *Salmo fario*, *Rana temporaria*, and *Amblystoma tigrinum*. This work was done in the Department of Zoology and Comparative Anatomy of the Oxford University Museum, where I had the privilege of using the admirable series of developmental stages belonging to the late Dr. J. W. Jenkinson and to Professor E. S. Goodrich, F.R.S., as well as my own. For purposes of illustration I have had recourse to photomicrograms, for in a study of this nature, which consists largely in a hunt for mesenchymal condensations, drawings by hand are both more laborious and less satisfactory. I wish to express my gratitude to my colleague Mr. J. Z. Young, of Magdalen College, for the assistance which he has kindly and willingly afforded me in the preparation of these photomicrograms, and to Professor Goodrich I would like to acknowledge my indebtedness for his helpful criticism and encouragement.

2. OBSERVATIONS.

(i) *Selachii*.

Since the trabeculae are said to arise in connexion with the maxillary processes, it seemed advisable at the outset to investigate the origin of these maxillary processes, and to obtain a clear picture of the geography of the region in which the trabeculae will make their appearance.

Fig. 1, Pl. 40, is of a median longitudinal section through an embryo of *Scyllium* at stage J, for comparison with fig. 2 (on the same plate), which is a similar section of an embryo 26 mm. in length, corresponding roughly to stage O. The chief point to notice is that in the latter the hypophysial cavity is much longer and deeper, and there is a considerable quantity of mesenchyme ventroposterior to it. Just in front of the opening of the hypophysial sac there is also more mesenchyme than at the previous stage, with the result that the fore-brain in this region is farther removed from the superficial epidermis. The question is, where has this mesenchymatous

tissue come from? This matter is not easy to determine with certainty, but there is little doubt that it is contributed largely from each side. Fig. 3, Pl. 40, is of a transverse section, which, owing to the cranial flexure, cuts the ventral surface of the head tangentially. This section and that shown in fig. 4, Pl. 40, are from embryos at stage M. The sides of the hypophysial sac are indicated by a thickening of the epidermis, and they are still relatively far apart. Immediately lateral to them can be seen a heaping-up of mesenchyme which gives the appearance of extending towards the middle line, which would, of course, have the effect of narrowing the hypophysial sac and carrying its aperture farther forward. This can be seen in fig. 4, Pl. 40, which is of a section horizontal to the body, and therefore transverse to the head. From both figs. 3 and 4, Pl. 40, it is obvious that the mesenchyme which grows in towards the middle line in this manner is related to the mandibular visceral arch, which latter is identified among other things by the plate of visceral muscle and the mandibular blood-vessel which will eventually become the efferent pseudobranchial artery. At the same time, it is clear that no proliferations of mesenchyme from the premandibular or any other somite take part in the formation of the maxillary processes.

Eventually the two maxillary processes meet beneath the forebrain, and, in front of the aperture of the hypophysial sac, a median raphé is discernible in the mesenchyme. Behind the aperture of the hypophysial sac no such raphé is visible, which means that there has been a growth forwards of mesenchyme beneath the hypophysial sac, accompanying the ingrowth of the maxillary processes.

The result of this preliminary investigation is, therefore, to confirm the accounts given of the origin of the maxillary process and deepening of the hypophysial sac by overgrowth given by Woerdeman (1914) and Haller (1923), and to justify to a certain extent Allis's (1923) contention that the hypophysial pit is 'the result of the folding or rolling together of two ectodermal surfaces and their fusion with each other excepting along the median line'. Allis contends that the hypophysial pit arises by no invagination at all, but by overgrowth. In a matter of this kind

and where there are no immutable fixed points it is always difficult to decide how much of a pit is due to invagination and how much to overgrowth. That overgrowth plays a large part in the formation of the hypophysial sac in *Selachii* is clear, but it seems hard to deny to invagination any part of its formation, especially as in other forms such as the *Teleostei* and *Amphibia* there is no doubt of its development as a definite ingrowth, albeit solid. This matter has, however, no direct bearing on the problem at issue, and the conclusion so far arrived at is that mesenchyme derived from the mandibular visceral arch finds its way to a position on each side of the hypophysial sac, between the floor of the forebrain and the epidermis of the ventral side of the head.

Fig. 5, Pl. 41, is of a transverse section passing tangentially through the ventral side of the head in an embryo 23 mm. in length, just cutting the floor of the forebrain and the optic nerve. On each side of the forebrain can be seen a mesenchymatous condensation in the maxillary process, and close examination reveals the fact that it is a double condensation, consisting of the rudiment of the palatine process of the pterygo-quadrates externally, and of the rudiment of the trabecula internally. At an earlier stage (19 mm.) these two rudiments are indistinguishable, and at 23 mm. they are still intimately connected by mesenchyme a little less dense. It is important to notice that these rudiments, of the palatine process of the pterygo-quadrates as well as of the trabecula, are condensations *in situ* of the mesenchyme of the maxillary process; their cells are continuous by gradual transition with the cells of the remainder of the mesenchyme, and there is no evidence of proliferation from any other source. Fig. 6, Pl. 41, showing a section transverse to the head, is from an embryo 23 mm. long but slightly more developed than the previous one, and gives the relations of the trabecular rudiment to the optic nerve. The rudiment of the inferior oblique muscle derived from the premandibular somite is present, but it enters into no relation with the trabecular rudiment.

Fig. 7, Pl. 41, is of a paramedian longitudinal section through an embryo 25 mm. long, showing the relations of the trabecular

rudiment to the optic nerve and other structures in the head. The connexion between the trabecula and the palatine process of the pterygo-quadrates is still evident, and it is also clear that if the side of the mouth (as cut in the section) be regarded as occupying the position of a mandibular visceral cleft, the trabecular rudiment occupies a position serially comparable with that of the skeletal elements of the mandibular and posterior visceral arches.

Fig. 8, Pl. 41, is also from a 25 mm. embryo, and it shows that the trabecular rudiment and the palatine process of the pterygo-quadrates are now more distinct. The rudiment of the polar cartilage is also present. This element has been shown (this volume, p. 591) to chondrify in contact with the foremost part of the parachordal, and the present investigation indicates that its procartilaginous rudiment also arises in that position. I have not been able, however, to satisfy myself from the material at my disposal that this polar cartilage rudiment arises from the mandibular arch, probable though it may be.

Fig. 9, Pl. 42 (26 mm. stage) is not much more advanced than the previous one; the trabeculae extend farther backwards (upwards in appearance, owing to the cranial flexure) without connecting with the polar cartilage rudiments, and the parachordals are now definitely cartilaginous.

Fig. 10, Pl. 42, shows the relations of the trabeculae in a section transverse to the head of an embryo 26 mm. long, cut slightly obliquely. The trabeculae are some distance beneath the dura mater, and are connected to one another by a thin strand of mesenchyme which includes the disappearing duct of the hypophysial sac. Figs. 11 and 12, Pl. 42, are of transverse sections of an older embryo, 29 mm. long, in which the trabeculae are now definitely cartilaginous, continuous with the polar cartilages and parachordals, and forming the floor of the prechordal part of the brain-case. Except in the region of the sub-pituitary space, the trabeculae have now approximated more closely to the dura mater, although still lying outside it.

Summing up, therefore, the evidence obtained from a study of the Selachii, the following points lend support to the view that the trabeculae are visceral structures:

The origin of the maxillary processes from the visceral mesenchyme associated with the mandibular arch;

The origin of the trabeculae as condensations in situ in the maxillary processes;

The community of origin of the mesenchymatous condensations of the rudiments of the trabeculae and of the palatine processes of the pterygo-quadrates;

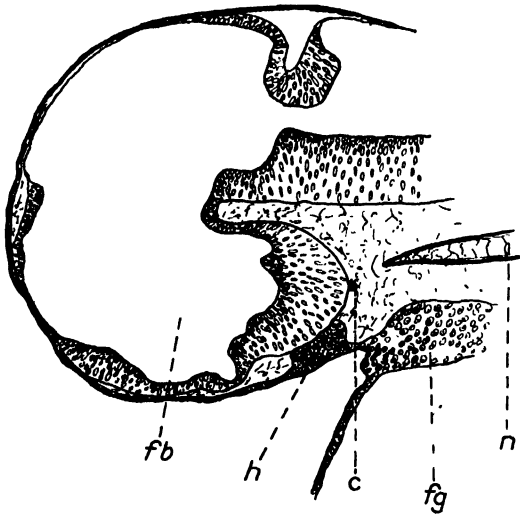
The absence of any evidence of proliferation from the pre-mandibular somite.

(ii) Teleostei.

In *Salmo fario* the conditions of the origin of the maxillary processes are more difficult to determine; for one thing the hypophysis arises as a solid ingrowth, and it is, therefore, not possible to estimate the relative importance of invagination and overgrowth as in the Selachii; for another, the notochord does not extend so far forward as in other forms, and its anterior end appears to be undergoing reduction. This is evident from Text-fig. 2. The position of the transverse commissure connecting the premandibular somites of Selachii and of *Amia* (de Beer, 1924) is indicated by a strand of mesenchyme continuous with the rudiments of the eye-muscles and extending inwards to a point on the hind-wall of the infundibulum. The gap between this point and the tip of the notochord at this stage is probably not unconnected with the subsequent formation of the myodome.

On each side of the ventral surface of the head, lateral to the hypophysis and median to the eyes, there is a plaque of thickened mesenchyme, in close contact with the epidermis. Posteriorly these plaques are continuous with the mesenchyme of the mandibular arch, as may be seen in fig. 14, Pl. 43, which is of a transverse section (trout 25-2) cut slightly obliquely, and showing the angle of the lower jaw on the right side. From the inner side of each of these mesenchymatous plaques, at about the level of the hypophysis, the trabeculae are proliferated inwards, upwards, and backwards, as shown in fig. 13, Pl. 43 (trout 24-2). Posteriorly, the mesenchyme of the trabecular rudiment becomes less dense, and it comes into contact with

the strand of mesenchyme which, as described above represents the transverse commissure between the two premandibular head cavities. It might be supposed that this contact was evidence for the origin of the trabeculae from the transverse commissure and so from the premandibular somite. But the connexion of the trabecular rudiment with the mesenchymatous plaque is so dense and intimate, while that with the transverse



TEXT-FIG. 2.

Median longitudinal section through the head of an embryo of *Salmo fario* (trout 23) showing the relation of the hypophysis, notochord, and transverse commissure between the premandibular somites.

commissure is so ephemeral, that there can be little doubt that it is from the former and not from the latter that the trabeculae are formed. There is no connexion whatever between the mesenchymatous plaque and the transverse commissure of the premandibular somites, and at an earlier stage (trout 24-1), in which the trabeculae are not yet present, there is no suggestion that they will arise from the transverse commissure. It is further to be noticed that the orientation of the nuclei in the

transverse commissure and in the trabecular rudiments is different. If the transverse commissure represents the acrochordal of other forms, there would be nothing surprising in the fact that the hinder ends of the trabeculae came into contact with it.

Another point of interest is the distance which separates the trabecular rudiments from the floor of the brain or infundibulum, as is evident from all the figures on Pl. 43.

Fig. 15, Pl. 43, shows the conditions as seen in a paramedian longitudinal section (trout 23 long.). Here, again, it is clear that if the angle of the mouth is regarded as occupying the position of a former mandibular visceral cleft, the mesenchymatous plaque from which the trabecular rudiments arise is in the correct position to represent a premandibular visceral arch.

Fig. 16, Pl. 43, is of a transverse section through an embryo slightly older than the foregoing (trout 25 A). The trabeculae are just cartilaginous, and they are still connected by a strand of dense tissue with the mesenchyme of the mandibular arch. This strand is a remnant of the mesenchymatous plaque of the previous stages, and its interest lies in the fact that in it the rudiments of the palatine processes of the pterygo-quadrates will make their appearance. At the present stage, however, they are absent, and the only skeletal structures of the palate are the trabeculae.

Fig. 17, Pl. 43, is from a still older embryo (trout 26-1); the trabeculae are at a considerable distance from the floor of the infundibulum, and the connecting strand is still evident. In fig. 18, Pl. 43 (trout 29 II), which is of a section slightly farther forward than that of fig. 17, the trabeculae are seen approximating to one another in a manner which will eventually give rise to the trabecula communis, and the palatine process of the pterygo-quadrates has appeared in the connecting strand.

The evidence provided by the Teleostei concerning the origin of the trabecula leads, therefore, to the same conclusion as that provided by the Selachii, and largely for the same reasons. Although the hinder ends of the trabeculae soon come into relation with the transverse commissure of the premandibular somites, the evidence points to their origin from a mesenchy-

matous plaque, itself related to the mandibular arch and from which the palatine process of the pterygo-quadrate will eventually arise. The trabeculae in the trout are even more aloof from the dura mater than they are in *Scyllium*.

(iii) *Anura*.

The first appearance of the rudiments of the trabeculae in *Rana temporaria* is shown in fig. 19, Pl. 44, and they take the form of slender condensations of mesenchyme parallel with the pterygo-quadrate and half-way between the latter and the floor of the forebrain (frog AA 2). These rudiments are nothing but faint condensations in situ of the mesenchyme, and they enter into no relations with the derivatives of the premandibular somites. Far forward, in the region of the upper lip, are the rudiments of the suprarostrals, and, as Spemann (1898) emphasized, they are independent of the trabeculae at this stage.

The trabeculae soon enter into relations with the pterygo-quadrate, and fig. 20, Pl. 44, is of a section through a frog embryo (frog AA 3) passing through the commissura quadratocranialis anterior of Gaupp's nomenclature. The condensations of the trabecular and pterygo-quadrate rudiments are here seen to be continuous. Fig. 21, Pl. 44, is from the same embryo, at a more posterior level, and shows the relation of the trabeculae to the optic nerve and the floor of the forebrain. The same is shown in horizontal section in fig. 22, Pl. 44 (frog II B). Posteriorly, the trabecular rudiments are now continuous with the 'Balkenplatte' of Stöhr's (1882) description, which probably represent the foremost parts of the parachordals. Anteriorly, the trabecular rudiments connect with those of the suprarostrals. This is also shown in fig. 23, Pl. 44, which is of a transverse section (frog B T). The suprarostrals were considered by Stöhr (1882) to represent the foremost portions of the trabecular bars, but Spemann (1898) contests this view on the grounds that procartilaginous continuity between two elements is no proof of their genetic affinity. The question is difficult to decide, and the suprarostrals seem to be structures *sui generis* developed in relation to the conditions of the larval mouth of the tadpole. That the suprarostal elements are visceral structures there can

be no doubt owing to their position and their mode of origin, far in front of the premandibular somites. That they should be connected with the trabeculae by procartilage may, as Spemann says, be no proof of their affinity, but it leads to the suspicion that the trabeculae have visceral affinities. It may be said that the trabeculae become intimately connected with the parachordals, and that, as the latter are certainly not visceral structures, the criterion of connexion means nothing. But it must be remembered that, at early stages of development, the trabeculae are not connected with the parachordals by procartilage.

Fig. 24, Pl. 44, is from the same embryo as fig. 23, Pl. 44, but the section is taken more posteriorly. The trabeculae are now definitely cartilaginous and are pressed close against the dura mater, a position which they did not previously occupy. On comparing fig. 24, Pl. 44, with figs. 19 or 21, Pl. 44, it would seem that the connexion of the trabeculae with the dura mater is due to the expansion of the brain and its overlying dura mater, the trabeculae remaining relatively passive.

The evidence provided by the *Anura* concerning the method of origin of the trabeculae cannot be regarded as conclusive, for the conditions do not easily lend themselves to a determination of the origin of the mesenchyme of the maxillary process. But the origin of the trabecular rudiments *in situ* in this mesenchyme, in a manner identical with that of the pterygo-quadrate, their relations with the pterygo-quadrate in the form of the commissura quadratocranialis anterior, and their relations with the suprarostrals, argue in favour of their visceral nature.

(iv) *Urodela*.

In *Amblystoma* the trabecular rudiments are first found in the form of elongated mesenchymatous condensations running forward beneath the optic nerves and median to the eyes and nasal sacs. Fig. 25, Pl. 45, is of a horizontal section (*Axolotl* A I) in which the rudiment is just discernible, and all that can be said of it is that it constitutes a very slightly condensed region of the general mesenchyme of the head.

Fig. 26, Pl. 45 (*Axolotl* B. T. I.), is of an embryo slightly more

developed, and the trabecular rudiments take the form of rods the diameter of which is made up of three or four cells. The interest of this specimen lies in the fact that the rudiments can be seen to be continuous with the adjacent mesenchyme, and in many cases it is hard to say whether any given cell forms part of the trabecular rudiment or not. Here, again, therefore, there is evidence that the trabecular rudiments arise as condensations in situ, and it is necessary to conclude that the source from which the trabeculae arise has also produced all the mesenchyme of this region of the head. Since there is no reason to believe that this mesenchyme has been produced by the sclerotomes of the mesodermal somites, it must be inferred that the trabeculae are not formed from the somites either.

Fig. 27, Pl. 45 (Axolotl Aoo II), is of a longitudinal paramedian section showing the relations of the mesenchyme from which the trabecular rudiments arise to the optic nerve and to the mouth and visceral arches. Fig. 28, Pl. 45, is from a slightly older embryo in which chondrification has just set in (Axolotl h. L), and by studying the section of this embryo it can be seen that the trabeculae do not lie in a perfectly straight line in front of the parachordals, but dip downwards as they run forwards, thus lying more or less parallel with the general trend of the visceral arches.

Fig. 29, Pl. 45 (Axolotl B. J. K.), is a transverse section through an older embryo showing how the trabeculae acquire relations with the dura mater by the infundibulum dipping down between them, as in the frog. There is in *Amblystoma* no palatine process of the pterygo-quadrate, and therefore the trabeculae are the only cartilaginous skeletal structures in the palate.

On the whole, the evidence from *Urodela* confirms Platt's view that the trabeculae are not derived from the sclerotomes, but arise from mesenchyme which can hardly be anything other than visceral.

3. DISCUSSION.

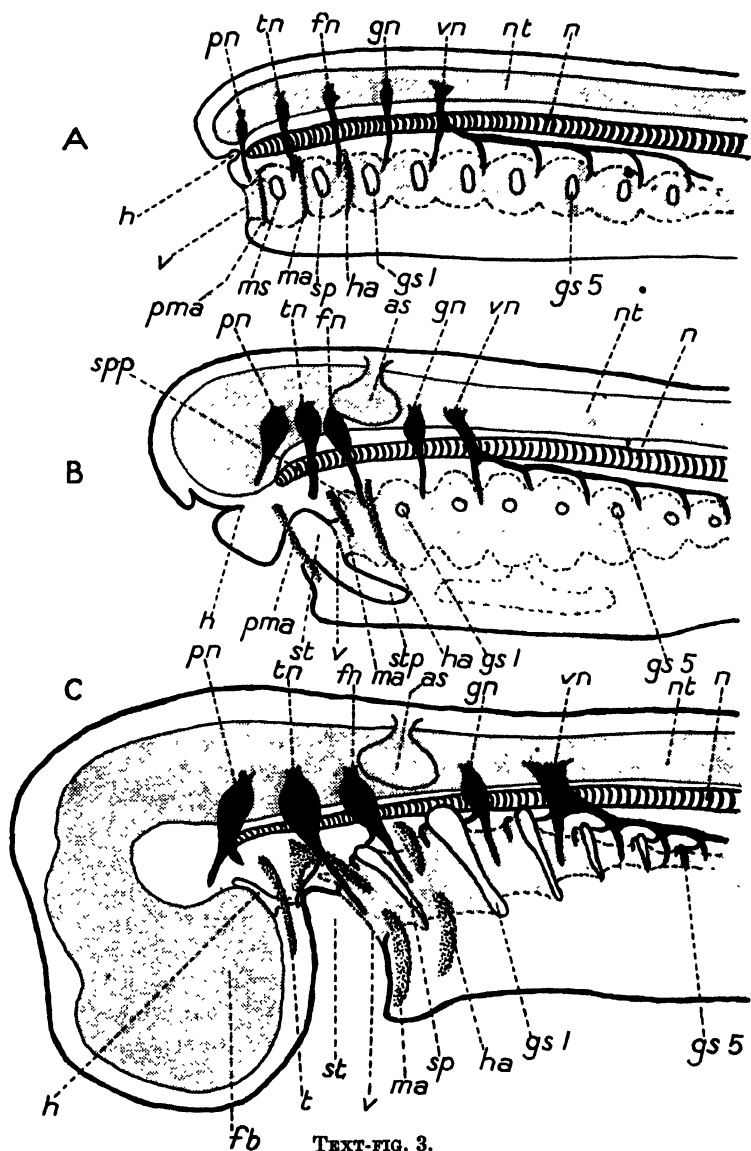
The evidence derived from a study of the four types described in this paper provides a convergence of results which accords

well with the view that the trabeculae are not derived from sclerotomes, but from visceral mesenchyme, belonging presumably to the premandibular arch. It now remains to consider some of the implications of this view.

If the trabeculae (and by this term is meant the trabeculae *sensu stricto*, for it has not been possible to come to definite conclusions regarding the polar cartilages) represent part of the cartilaginous skeleton of the premandibular arch, important changes must have taken place with regard to the mouth and the anterior region of the gut in those forms in which the trabeculae contribute to the formation of the brain-case. Originally the trabeculae must, on the hypothesis that they are visceral arches, have surrounded the anterior end of the gut, and the mouth opening must have passed backwards, as it were, between their legs. It may be imagined that there were ventral elements in the premandibular arch, corresponding serially to Meckel's cartilage and the ceratohyal.

Behind the trabeculae or premandibular arch there must have been a mandibular visceral cleft to separate the premandibular from the mandibular arches, and this cleft must have been related to the pre- and post-trematic branches of the nerve of the mandibular arch (the maxillary and mandibular branches of the trigeminal nerve) exactly as the spiracle is related to the pre- and post-trematic branches of the facial nerve, and the gill-slits are related to the glossopharyngeal and vagus nerves. The profundus nerve would then be the nerve of the premandibular arch, which is very appropriate, since its corresponding ventral root (the oculomotor) innervates the premandibular somite, and this profundus nerve would not be expected to divide into pre- and post-trematic branches, for there was no visceral cleft for it to fork over. The dorsal ends of the skeletal elements of the premandibular and following visceral arches may be regarded as having been more or less firmly attached to the axial skeleton in the form of the notochord and its sheaths.

An idea of this state of affairs may be obtained from a consideration of the conditions which obtain in *Amphioxus*, although that animal is considerably modified from what the original chordate type must have been. It is to be noted that



TEXT-FIG. 3.

Diagram showing the relations of the mouth, visceral arches, visceral pouches and clefts, and dorsal nerve-roots in *A*, hypothetical ancestral chordate; *B*, Ammocoete larva of *Petro-myzon*; and *C*, embryo of *Scyllium*, to illustrate the theory that the trabeculae are premandibular visceral arches.

the mouth in the adult *Amphioxus* is a perforation through a vertical membrane, the velum, and that it leads into the foremost part of the gut; i.e. there is no pre-oral gut. Added to this it must be noted that the neural tube of *Amphioxus* does not extend farther forward than the position of the neuropore. The extreme anterior extension of the notochord must be associated with the secondary formation of the anterior fin, since originally the notochord cannot have extended beyond the morphological anterior end of the body, represented by a point close to that of the neuropore. The relations of the essential features of the hypothetical ancestral chordate are shown in Text-fig. 3 A.

The origin of the Craniates, and particularly of the Gnathostomes, was accompanied by the formation of a large and expanded anterior region of the neural tube, giving rise to the brain. This expansion took place in a remarkable way, for the position of the neuropore remained close to the hypophysis and the tip of the notochord, and it was the dorsal part of the neural tube, behind the neuropore, which bulged forwards and downwards, eventually coming to lie in front of the point of closure of the neuropore, giving rise to the forebrain. Practically speaking, the neural tube became U-shaped, with the concavity ventral, forming the *plica encephali ventralis*.

The formation of the forebrain, and especially of the telencephalon, in this manner must have resulted in the displacement downwards and backwards of the mouth opening. The stomodaeum, instead of being anterior and opening straight back between the premandibular arches, would come to be situated on the ventral side of the head. It would no longer open into the extreme anterior end of the gut, but slightly farther back and beneath this foremost portion, which would then become what is known as the pre-oral gut. The skeletal elements of the premandibular arch would no longer lie astride the mouth opening, for the latter would have been withdrawn from under them; they would lie on the anterior dorsal wall of the stomodaeum, behind and beneath the newly formed telencephalon. The dorsal portions of the skeletal elements of the premandibular arch would thus become the *trabeculae cranii*, while the ventral

elements would presumably disappear. These relations are shown in Text-fig. 3 c.

By this ventral and posterior displacement of the stomodaeum the hypophysial sac would be rotated so that it pointed upwards, between the trabeculae. Another important result of this displacement would have been the approximation of the mouth opening to the mandibular visceral clefts, for the latter would no longer be separated from the former by a visceral arch, the stomodaeum having slipped beneath and behind the premandibular arch. It might be supposed that the mouth actually became confluent with the mandibular clefts, and there would then be an explanation of the fact that the lateral portions of the mouth of *Gnathostomata* (the 'angles') bear relations to the trigeminal nerve which suggest, as Johnston says (1907, p. 22), that the maxillary and mandibular branches represent pre- and post-trematic rami of a branchial nerve. But it must be remembered that the maxillary and mandibular branches of the trigeminal nerve differ from a typical branchial nerve in that they lack the visceral sensory component. A more satisfactory explanation is that in the *Gnathostomata* the mouth has extended sideways and upwards behind the pre-mandibular arch, obliterated the mandibular visceral pouch and cleft, and occupied its position. With the disappearance of the essential feature of a visceral cleft—the endodermal mandibular pouch—the visceral sensory component of the trigeminal nerve would have disappeared also, leaving the general cutaneous and visceral motor components, forking as pre- and post-trematic rami round the angles of the mouth, which angles have usurped the position of the former mandibular cleft.

It may be mentioned that Dohrn (1882, p. 255) claimed to find representatives of a pair of visceral clefts in the mouth of certain fish. He was concerned with the since-abandoned problem of finding for the vertebrates a mouth different from that of their then-supposed Annelid ancestors. He stated that in *Gobius*, *Hippocampus*, and *Belone* there was a pair of endodermal pouches extending towards the skin from the fore-gut, in front of the hyoid or spiracular pouches, and that the mouth first became perforated on each side in connexion

with these 'mandibular' pouches, and subsequently became perforated in the middle line. A similar state of affairs was reported by Platt (1891) for *Batrachus tau*. More recently, a pair of prespiracular endodermal pouches has been described in *Lepidosteus* by Veit (1924) and van Schrick (1927). But these pouches subsequently become reduced, and absorbed into the hyoid pouches. The presence of these pouches is of great interest, and their reduction and disappearance supports the view that the mouth of the *Gnathostomata* has obliterated them, by extending laterally, between the pre-mandibular arch in front and the mandibular arch behind. It is interesting to note that Platt (1891) considered the conditions of the formation of the mouth in *Squalus* as 'indicating a possible reduction of gill-clefts which once belonged to the anterior region of the head'. In this manner it can be understood how the mouth became able to bite, by the approximation of its anterior and posterior borders as upper and lower jaws. The lower jaw is constituted by the mandibular arch, the upper jaw strictly by the premandibular.

It may now be asked what light a consideration of the *Cyclostomata* throws on the foregoing argument. In *Petromyzon* Koltzoff (1901) showed that the sclerotomes of the first three somites of the head contributed to the formation of the structures which he, following Parker (1888), called the trabeculae. These observations were confirmed by Filatoff (apparently unpublished, but quoted by Sewertzoff, 1916). But Sewertzoff (1916) has contended that the structures in the *Ammocoete* larva which Parker and subsequent authors had called trabeculae are really the anterior portions of the parachordals of *Gnathostomata*, and that, consequently, the brain-case of *Petromyzon* is entirely chordal and sclerotomic in composition. He bases this contention on the relations of the cartilages in question to the notochord; the brain, and the hypophysis (1917, p. 480 et seq.). An examination of my material of *Petromyzon* has shown me that at a stage $4\frac{1}{2}$ mm. in length, in which there is very young cartilage, the so-called trabeculae are perfectly continuous without suture of any kind with the cartilage which flanks the notochord closely

and which is obviously parachordal. Dr. Tribe and Dr. Wyeth, who are investigating the development of the skull of *Petromyzon*, have kindly permitted me to mention that in the material which they have studied they have made the same observation. I am therefore inclined to accept Sewertzoff's opinion that the so-called trabeculae of *Petromyzon* are really parachordals, and that the brain-case of this animal, being entirely chordal in composition, contains no trabeculae. This being so, if the trabeculae are the premandibular visceral arches, and the brain-case of *Petromyzon* does not contain them, the trabeculae in *Petromyzon* should retain their original relations as premandibular visceral arches. Now there is in the *Ammocoete* a pair of bars of muco-cartilage which correspond exactly to the required premandibular arches. Sewertzoff (1916) called them the third prebranchial arches, the first prebranchial being the hyoid and the second prebranchial the mandibular arches. These arches are shown in figs. 30 and 31, Pl. 46.

These premandibular arches of *Petromyzon* lie astride the stomodaeal invagination and close to its side-wall and roof; their dorsal ends extend to a point just beneath the front ends of the parachordals (trabeculae of Parker) to which they ultimately become attached (just as in *Gnathostomata* the trabeculae become attached to the parachordals) forming the anterior attachment of the subocular bar of the adult; they lie in a position corresponding serially to that of the remaining visceral arches. Sewertzoff attempted to recognize in the adult *Petromyzon* a number of other prebranchial visceral arches in front of that which is here called the premandibular, but his evidence is insufficient. It can scarcely be without significance that in the *Ammocoete* larva the arch which is here called the premandibular is the most anterior of all: that the nerve related to it or its supposed homologue the trabecula (the profundus nerve) is the most anterior dorsal nerve-root; and that the ventral nerve-root (the oculomotor) corresponding to the profundus innervates a somite (the premandibular) which is believed with good reason to be the most anterior of the whole series. In fact, the recognition of a premandibular visceral arch

fits in perfectly with all the other evidence of chordate cephalogeny, and it would seem that the trabecula is the skeleton of that arch. But there is no room on the evidence for any arches anterior to the premandibular, and the structures which Sewertzoff has called the fourth and fifth prebranchial arches must be structures associated with the very specialized development of the upper lip and sucking mouth of the present-day *Cyclostomata*.

The skeletal elements of *Petromyzon* are, then, better rather than less well understood on the view that the trabeculae of *Gnathostomata* represent premandibular visceral arches. The conditions in the *Ammocoete* are shown in Text-fig. 3 B. Attention may now be turned to the mouth and related structures of *Petromyzon*. The *Ammocoete* larva possesses a velum which, like that of *Amphioxus*, must be regarded as the remains of the oral plate perforated by the mouth opening when communication is established between the fore-gut and the stomodaeal invagination. The position of the velum will of course depend on that of the stomodaeum, and if the latter is a deep invagination the velum will be situated farther back than if the stomodaeum were only a shallow pit. In *Petromyzon* the stomodaeum is extraordinarily deep, and it extends back into a pair of lateral pouches on each side of the velum and fore-gut, called by Sewertzoff the second prebranchial pouches. They are indicated in figs. 30 and 31, Pl. 46.

Immediately behind the velum is the pair of transient pouches which Dohrn (1886 and 1887) identified with the hyoid and described as giving rise to the peripharyngeal ciliated bands. But the fact that the first endodermal pouch behind the velum in *Petromyzon* is the hyoid is no evidence that the hyoid pouch was the most anterior in the ancestral chordate. A pair of mandibular pouches may have existed in front of the hyoid and become perforated to the exterior to form mandibular clefts. Indeed, Stensiø's (1927) reconstructions of Ostracoderms (which may be considered without necessarily accepting his interpretation of them) definitely show a pair of open mandibular clefts. In the history of *Petromyzon* it would seem that the mandibular pouches became reduced (as they do in *Lepidosteus*),

ceased to communicate with the exterior, and had their place taken by the extensive invagination of the stomodaeum. The depth of this invagination would necessarily result in a relatively depressed position of the velum. The mouth of *Petromyzon* would resemble that of the *Gnathostomata* if the lateral pouches of the stomodaeum (Sewertzoff's second prebranchial pouches) were perforated to the exterior confluent with the central mouth opening, behind the premandibular arch, thus giving rise to 'angles'.

The position of the velum in the Ostracoderms must of course be a matter of conjecture, but since the mandibular clefts of necessity presuppose a pair of mandibular endodermal pouches, the velum must have been situated anterior to them and to the clefts, and not posterior to them as suggested by Stensiø (1927, fig. 37, p. 347). This point has also been stressed by Goodrich (1931 A).

The Ammocoete larva possesses peripharyngeal ciliated grooves as does *Amphioxus*, but their comparison is a matter of almost indescribable complexity and confusion. Dohrn (1886 and 1887) claimed emphatically that the peripharyngeal ciliated grooves of the Ammocoete larva themselves were the remains of the transient hyoid visceral pouch. After examining my own fairly extensive material of early stages of development of *Petromyzon*, I am inclined to regard these grooves as developed in the posterior wall of and therefore behind the hyoid pouches, the latter being represented by the blind sacs shown in figs. 30 and 31, Pl. 46. But be this as it may, the peripharyngeal grooves of the Ammocoete either themselves are, or are behind, the hyoid visceral pouch. In *Amphioxus*, on the other hand, the peripharyngeal grooves pass in front of the internal opening of the club-shaped gland and of the first (transient) gill-slit of the primary series (Willey, 1891). However the gill-slits be considered—whether the club-shaped gland be considered an antimer to the first primary gill-slit or itself a primary gill-slit (see Goodrich, 1931 B), or whether the mouth of *Amphioxus* be regarded as a gill-slit (see van Wijhe, 1904)—the fact remains that the grooves cannot correspond perfectly in *Amphioxus* and in the Ammocoete

larva. It seems probable that the ciliated groove might arise running up any visceral arch, and in the *Ammocoete* it would seem to be on the hyoid arch. Judging by *Amphioxus*, the primitive position of the grooves must have been in front of the originally foremost visceral cleft (whichever that was), and therefore the position of these grooves in the *Ammocoete* provides no evidence that the hyoid visceral pouch was originally the most anterior of all.

4. SUMMARY.

1. The existing evidence concerning the origin and nature of the trabecula cranii is reviewed, and it is shown that it constitutes a *prima facie* case for supporting Huxley's opinion that it represents a visceral structure.

2. The origin of the trabecula is studied in *Scyllium canicula*, *Salmo fario*, *Rana temporaria*, and *Amblystoma tigrinum*, and the results of this investigation support Huxley's opinion.

3. The grounds for adhering to Huxley's view are chiefly that: the trabecular rudiment is a mesenchymatous condensation in the maxillary process; there is no evidence of the trabecular rudiment being derived from the somites; the trabecular rudiment is closely associated with that of the pterygo-quadrates; if the trabecular rudiment is of sclerotomic origin, then the palatine process of the pterygo-quadrates and the mesenchyme of the ventral side of the front of the head must also be derived from the sclerotomes: an impossible conclusion.

4. The implications of the recognition of the trabecula as a premandibular arch are considered, and it is concluded that the mouth of *Gnathostomata* represents the original velar perforation of *Amphioxus* which has extended to the side and obliterated a pair of mandibular clefts or the dermal pouches corresponding to them.

5. Sewertzoff's view that the brain-case of *Petromyzon* is wholly chordal in composition is supported, and the homologues of the trabeculae are represented by a pair of premandibular visceral arches.

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6. EXPLANATION OF PLATES 40 TO 46.

PLATE 40. All figures of *Scyllium canicula*.

- Fig. 1.—Longitudinal section, stage J.
Fig. 2.—Longitudinal section, 26 mm., stage O.
Fig. 3.—Transverse section, tangential to ventral surface of head, stage M.
Fig. 4.—Horizontal section, transverse to head, stage M.

PLATE 41. All figures of *Scyllium canicula*.

- Fig. 5.—Transverse section, tangential to ventral surface of head, 23 mm.
Fig. 6.—Horizontal section, transverse to head, 23 mm.
Fig. 7.—Longitudinal section, 25 mm.
Fig. 8.—Transverse section, tangential to ventral surface of head, 25 mm.

PLATE 42. All figures of *Scyllium canicula*.

- Fig. 9.—Transverse section, tangential to ventral surface of head, 26 mm.
Fig. 10.—Horizontal section, transverse to head, 26 mm.
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PLATE 43. All figures of *Salmo fario*.

- Fig. 13.—Transverse section (trout 24-2).
Fig. 14.—Transverse section (trout 25-2).
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Fig. 16.—Transverse section (trout 25 A).
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Fig. 18.—Transverse section (trout 29 II).

PLATE 44. All figures of *Rana temporaria*.

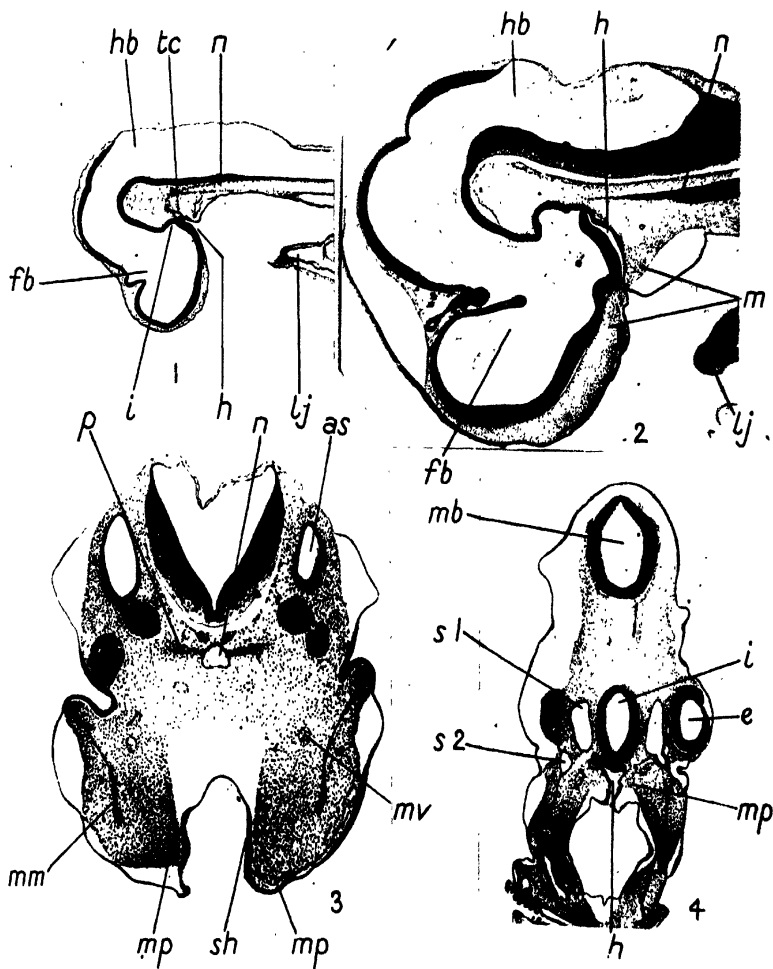
- Fig. 19.—Transverse section (frog AA 2).
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Fig. 22.—Horizontal section (frog II B).
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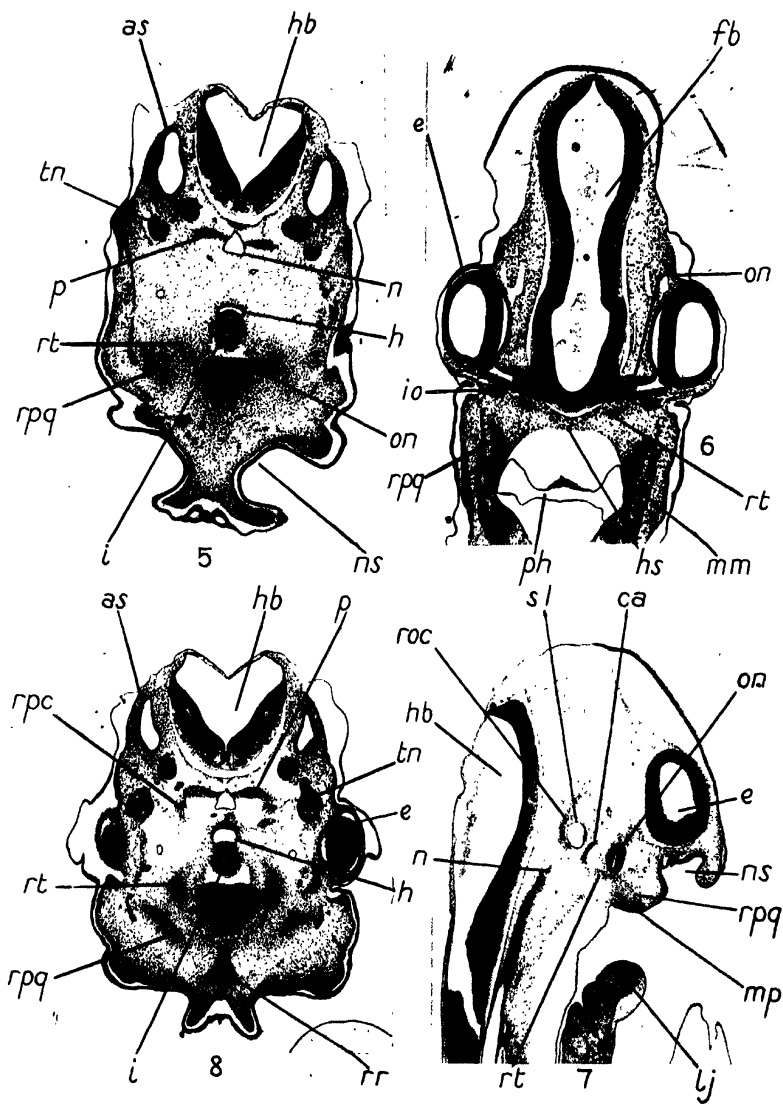
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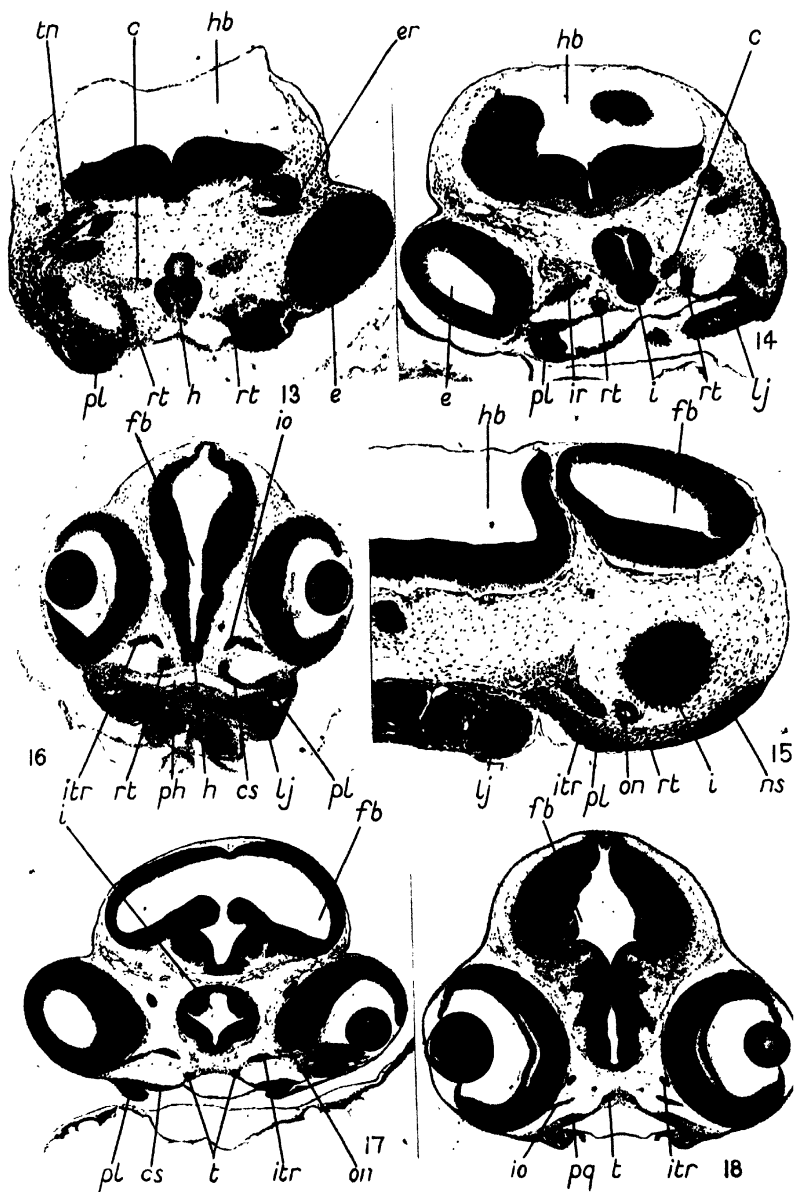
- Fig. 25.—Horizontal section (Axolotl A I).
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Fig. 27.—Longitudinal section (Axolotl Aoo II).
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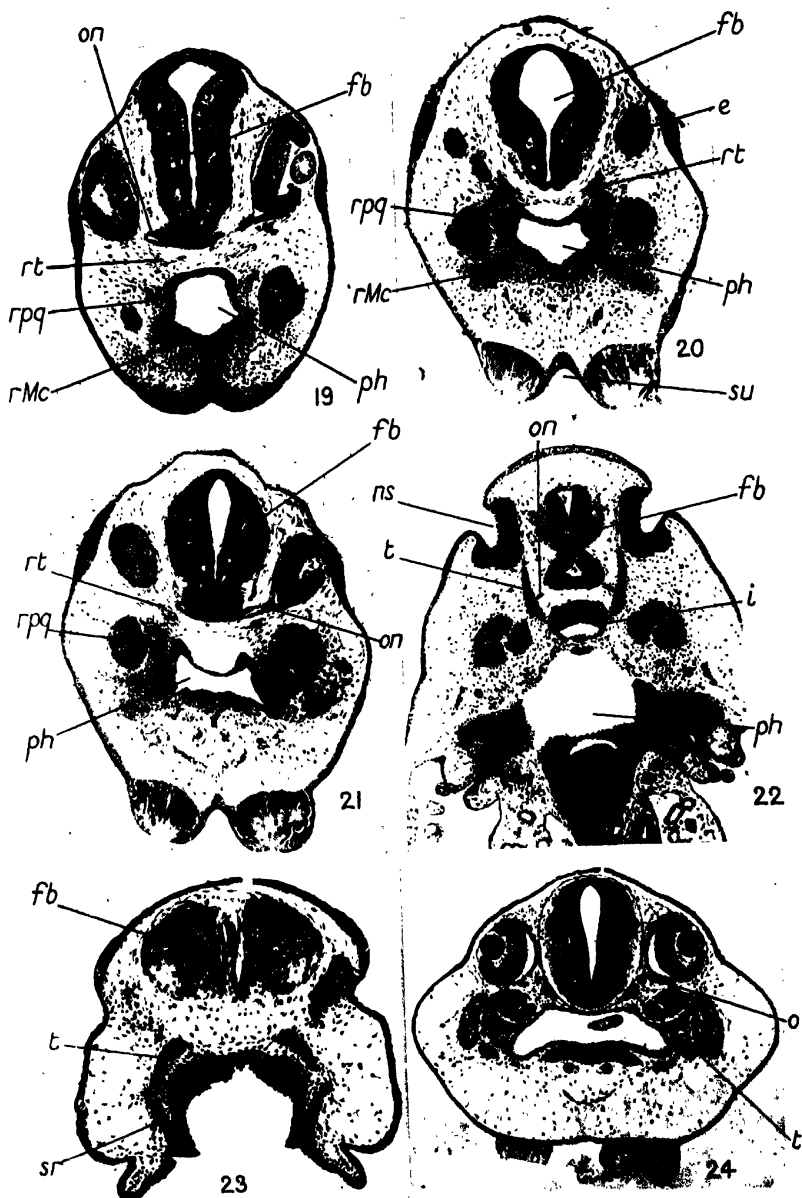
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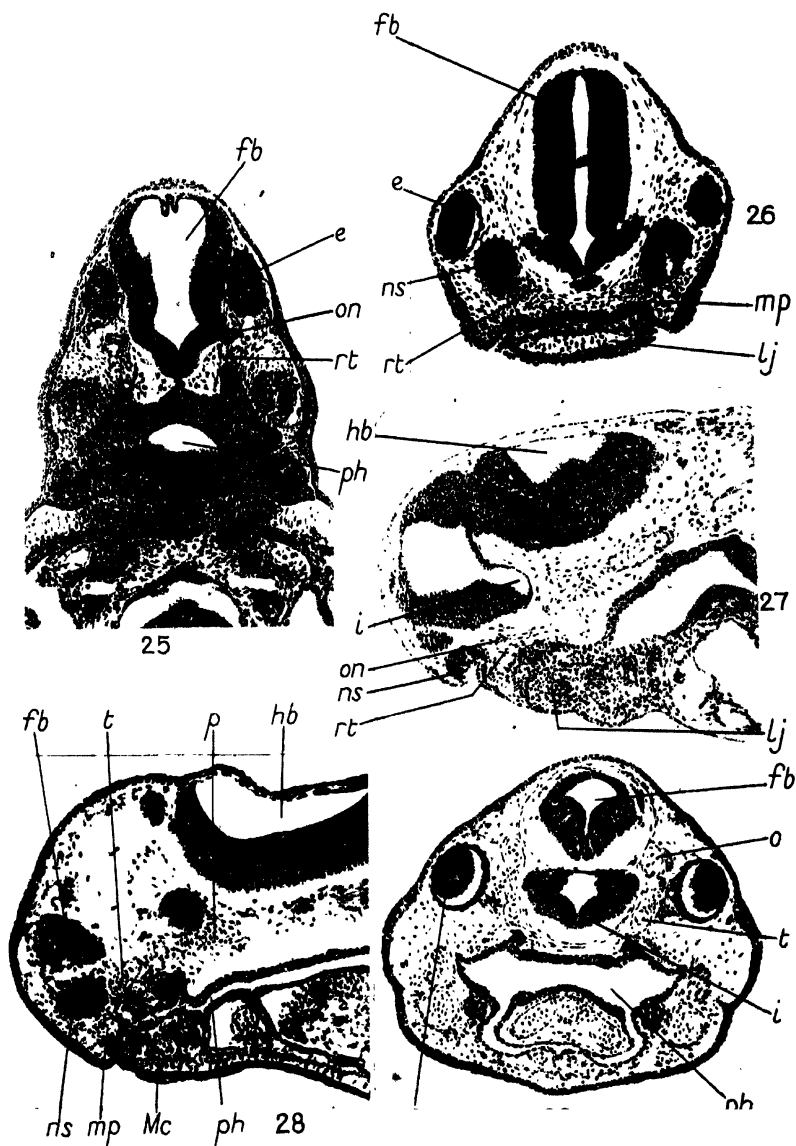
- Fig. 30.—Horizontal section.
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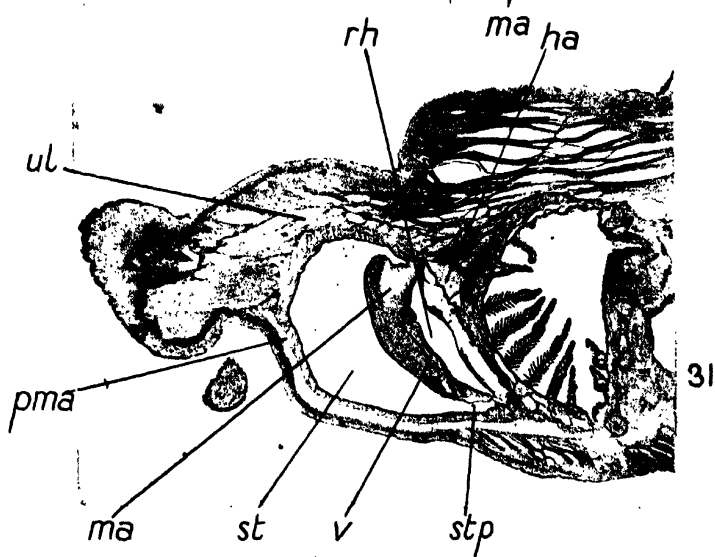
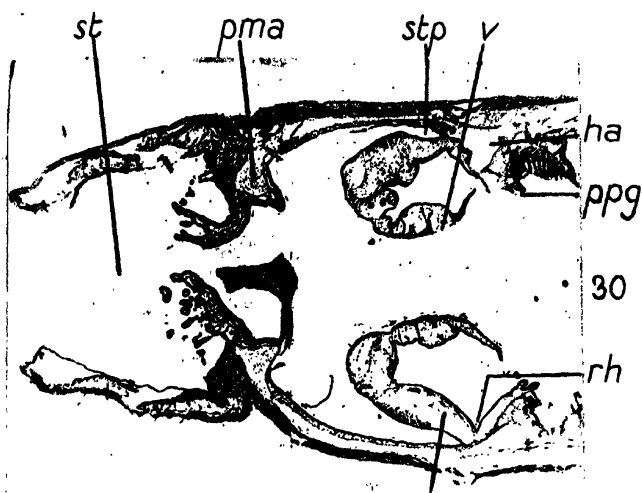












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